This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 9/50 — A1

(11) International Publication Number: WO 93/17669

(43) International Publication Date: 16 September 1993 (16.09.93)

(21) International Application Number: PCT/US93/01773

(22) International Filing Date: 1 March 1993 (01.03.93)

(30) Priority data:
843,485 28 February 1992 (28.02.92) US

(71) Applicant: BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West Seventh Street, Austin, TX 78701 (US).

(72) Inventors: HUBBELL, Jeffrey, A. ; 3006 Beverly Road, Austin, TX 78703 (US). PATHAK, Chandrashekhar, P. ; 3102 Stearns Hill Road, Waltham, MA 02154 (US). SAWHNEY, Amarpreet, S. ; 148 Church Street, Newton, MA 02158 (US). DESAI, Neil, P. ; 847 Alandele Avenue, Los Angeles, CA 90036 (US). HILL, Jennifer, L. ; 2501 Wickersham Lane, No. 821, Austin, TX 78741 (US).

(74) Agents: PABST, Patrea, L. et al.; Kilpatrick & Cody, 1100 Peachtree Street, Suite 2800, Atlanta, GA 30309-4530 (US).

(81) Designated States: AU, BB, BG, BR, CA, CZ, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHOTOPOLYMERIZABLE BIODEGRADABLE HYDROGELS AS TISSUE CONTACTING MATERIALS AND CONTROLLED-RELEASE CARRIERS

(57) Abstract

Hydrogels of polymerized and crosslinked macromers comprising hydrophilic oligomers having biodegradable monomeric or oligomeric extensions, which biodegradable extensions are terminated on free ends with end cap monomers or oligomers capable of polymerization and cross linking are described. The hydrophilic core itself may be degradable, thus combining the core and extension functions. Macromers are polymerized using free radical initiators under the influence of long wavelength ultraviolet light, visible light excitation or thermal energy. Biodegradation occurs at the linkages within the extension oligomers and results in fragments which are non-toxic and easily removed from the body. Preferred applications for the hydrogels include prevention of adhesion formation after surgical procedures, controlled release of drugs and other bioactive species, temporary protection or separation of tissue surfaces, adhering of sealing tissues together, and preventing the attachment of cells to tissue surfaces.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SK	Slovak Republic
CI	Côte d'Ivoire	KZ	Kazakhstan	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
CS	Czechoslovakia	LK	Sri Lanka	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	UA	Ukraine
DK	Denmark	MG	Madagascar	US	United States of America
ES	Spain	ML	Mali	VN	Viet Nam
FI	Finland	MN	Mongolia		

PHOTOPOLYMERIZABLE BIODEGRADABLE HYDROGELS AS TISSUE CONTACTING MATERIALS AND CONTROLLED-RELEASE CARRIERS

Field of the Invention

The present invention relates to photopolymerizable biodegradable hydrogels for use as tissue adhesives and in controlled drug delivery.

Background of the Invention

This is a continuation-in-part of U.S. Patent Application No. 07/843,485, filed February 28, 1992, entitled "Photopolymerizable Biodegradable Hydrogels as Tissue Contacting Materials and Controlled Release Carriers" by Jeffrey A. Hubbell, Chandrashekhar P. Pathak, and Amarpreet S. Sawhney.

Hydrogels as controlled-release carriers

Biodegradable hydrogels can be carriers for biologically active materials such as hormones, enzymes, antibiotics, antineoplastic agents, and cell suspensions. Temporary preservation of functional properties of a carried species, as well as controlled release of the species into local tissues or systemic circulation, are possible. Proper choice of hydrogel macromers can produce membranes with a range of permeability, pore sizes and degradation rates suitable for a variety of applications in surgery, medical diagnosis and treatment.

Adhesives and sealers

Fibrin gels have been used extensively in Europe as sealants and adhesives in surgery (Thompson *et al.*, 1988, "Fibrin Glue: A review of its preparation, efficacy, and adverse effects as a topical hemostat," *Drug Intell. and Clin. Pharm.*, 22:946; Gibble *et al.*, 1990, (1990), "Fibrin glue: the perfect operative sealant?" *Transfusion*, 30(8):741). However, they have not been used extensively in the United States

due to concerns relating to disease transmission from blood products. Synthetic polymers have been explored as adhesives (Lipatova, 1986, "Medical polymer adhesives," *Advances in Polymer Science* 79:65-93), but these materials have been associated with local inflammation, cytotoxicity, and poor biocompatibility.

Prevention of postoperative adhesions.

Formation of post-surgical adhesions involving organs of the peritoneal cavity and the peritoneal wall is a frequent and undesirable result of abdominal surgery. Surgical trauma to the tissue caused by handling and drying results in release of a serosanguinous (proteinaceous) exudate which tends to collect in the pelvic cavity (Holtz, G., 1984). If the exudate is not absorbed or lysed within this period it becomes ingrown with fibroblasts, and subsequent collagen deposition leads to adhesion formation.

Numerous approaches to elimination of adhesion formation have been attempted, with limited success in most cases. Approaches have included lavage of the peritoneal cavity, administration of pharmacological agents, and the application of barriers to mechanically separate tissues. For example, Boyers *et al.*, (1988) "Reduction of postoperative pelvic adhesions in the rabbit with Gore-Tex surgical membrane," *Fertil. Steril.*, 49:1066, examined Gore-Tex surgical membranes in the prevention of adhesions. For a review of adhesion prevention, see Holtz (1984) "Prevention and management of peritoneal adhesions," *Fertil. Steril.*, 41:497-507. However, none of these approaches has been cost effective and effective in *in vivo* studies.

Solutions of Poloxamer 407 have been used for the treatment of adhesions, with some success. Poloxamer is a copolymer of ethylene oxide and propylene oxide and is soluble in water; the solutions are liquids at

room temperature. Steinleitner *et al.* (1991) "Poloxamer 407 as an Intraperitoneal Barrier Material for the Prevention of Postsurgical Adhesion Formation and Reformation in Rodent Models for Reproductive Surgery," *Obstetrics and Gynecology*, 77(1):48 and Leach *et al.* (1990) "Reduction of postoperative adhesions in the rat uterine horn model with poloxamer 407, *Am. J. Obstet. Gynecol.*, 162(5):1317, examined Poloxamer solutions in peritoneal adhesion models and observed statistically significant reductions in adhesions; however, they were unable to eliminate adhesions, perhaps because of limited adhesion and retention on the injury site.

Oxidized regenerated cellulose has been used extensively to prevent adhesions and is an approved clinical product, trade-named Interceed TC7. This barrier material has been shown to be somewhat effective in rabbits (Linsky *et al.*, 1987 "Adhesion reduction in a rabbit uterine horn model using TC-7," *J. Reprod. Med.*, *32*:17; Diamond *et al.*, 1987 "Pathogenesis of adhesions formation/reformation: applications to reproductive surgery," *Microsurgery*, 8:103) and in humans (Interceed (TC7) *Adhesion Barrier Study Group*, 1989). It was shown to be more effective if pretreated with heparin, but was still unable to completely eliminate adhesions (Diamond *et al.*, 1991 "Synergistic effects of INTERCEED(TC7) and heparin in reducing adhesion formation in the rabbit uterine horn model," *Fertility and Sterility*, 55(2):389).

In summary, several lavage/drug/material approaches have been explored, but none of these approaches has been able to eliminate adhesions. An ideal material barrier would not evoke an adhesion response itself, stay in place without suturing (Holtz *et al.*, 1982 "Adhesion induction by suture of varying tissue reactivity and caliber," *Int. J. Fert.*, 27:134), degrade over a few weeks' time, effectively

reduce adhesions to very low extent, and be capable of delivering a drug to the local site of application for several days' time. None of the approaches developed and described to date meet these requirements.

Synthetic biodegradable polymers

The field of biodegradable polymers has developed rapidly since the synthesis and biodegradability of polylactic acid was first reported by Kulkarni *et al.*, 1966 "Polylactic acid for surgical implants," *Arch. Surg.*, 93:839. Several other polymers are known to biodegrade, including polyanhydrides and polyorthoesters, which take advantage of labile backbone linkages, as reported by Domb *et al.*, 1989 *Macromolecules*, 22:3200; Heller *et al.*, 1990 *Biodegradable Polymers as Drug Delivery Systems*, Chasin, M. and Langer, R., Eds., Dekker, New York, 121-161. Since it is desirable to have polymers that degrade into naturally occurring materials, polyaminoacids have been synthesized, as reported by Miyake *et al.*, 1974, for *in vivo* use. This was the basis for using polyesters (Holland *et al.*, 1986 *Controlled Release*, 4:155-180) of α-hydroxy acids (*viz.*, lactic acid, glycolic acid), which remain the most widely used biodegradable materials for applications ranging from closure devices (sutures and staples) to drug delivery systems (U.S. Patent No. 4,741,337 to Smith *et al.*; Spilizewski *et al.*, 1985 "The effect of hydrocortisone loaded poly(dl-lactide) films on the inflammatory response," *J. Control. Rel.* 2:197-203).

The time required for a polymer to degrade can be tailored by selecting appropriate monomers. Differences in crystallinity also alter degradation rates. Due to the relatively hydrophobic nature of these polymers, actual mass loss only begins when the oligomeric fragments are small enough to be water

soluble. Hence, initial polymer molecular weight influences the degradation rate.

Degradable polymers containing water-soluble polymer elements have been described. Sawhney et al., (1990) "Rapidly degraded terpolymers of dl-lactide, glycolide, and ϵ-caprolactone with increased hydrophilicity by copolymerization with polyethers," *J. Biomed. Mater. Res.* 24:1397-1411, copolymerized lactide, glycolide and ϵ-caprolactone with PEG to increase its hydrophilicity and degradation rate. U.S. Patent No. 4,716,203 to Casey et al. (1987) synthesized a PGA-PEG-PGA block copolymer, with PEG content ranging from 5-25% by mass. U.S. Patent No. 4,716,203 to Casey et al. (1987) also reports synthesis of PGA-PEG diblock copolymers, again with PEG ranging from 5-25%. U.S. Patent No. 4,526,938 to Churchill et al. (1985) described noncrosslinked materials with MW in excess of 5,000, based on similar compositions with PEG; although these materials are not water soluble. Cohn et al. (1988) *J. Biomed. Mater. Res.* 22:993-1009 described PLA-PEG copolymers that swell in water up to 60%; these polymers also are not soluble in water, and are not crosslinked. The features that are common to these materials is that they use both water-soluble polymers and degradable polymers, and that they are insoluble in water, collectively swelling up to about 60%.

Degradable materials of biological origin are well known, for example, crosslinked gelatin. Hyaluronic acid has been crosslinked and used as a degradable swelling polymer for biomedical applications (U.S. Patent No. 4,987,744 to della Valle et al., U.S. Patent 4,957,744 to Della Valle *et al.* (1991) "Surface modification of polymeric biomaterials for reduced thrombogenicity," *Polym. Mater. Sci. Eng.*, 62:731-735]).

Use of biodegradable materials for controlled drug release.

Most hydrophilic drugs are mechanically dispersed as suspensions within solutions of biodegradable polymers in organic solvents. Protein and enzyme molecular conformations are frequently different under these circumstances than they would be in aqueous media. An enzyme dispersed in such a hydrophobic matrix is usually present in an inactive conformation until it is released into the surrounding aqueous environment subsequent to polymer degradation. Additionally, some proteins may be irreversibly denatured by contact with organic solvents used in dispersing the protein within the polymer.

Polymer synthesis, degradation and local synthesis

Rapidly-degrading polymers currently suggested for short-term macromolecular drug release may raise local concentrations of potentially hazardous acidic degradation byproducts. Further, all biodegradable synthetic polymers reported thus far can only be processed in organic solvents and all biodegradable polymers are synthesized under conditions which are not amenable to polymerization *in vivo*. Thus, it has not been possible to make implantable materials as precisely conformed barriers, shaped articles, or membranes capable of delivering bioactive materials to the local tissue.

It is therefore an object of the present invention to provide hydrogels which are biocompatible, biodegradable, and can be rapidly formed by polymerization *in vivo*.

It is a further object of the present invention to provide a macromer solution which can be administered during surgery or outpatient procedures and polymerized as a tissue adhesive, tissue encapsulating medium, tissue support, or drug delivery medium.

It is a still further object of the present invention to provide a macromer solution which can be polymerized *in vivo* in a very short time frame and in very thin, or ultrathin, layers.

Summary of the Invention

Disclosed herein are biocompatible, biodegradable, polymerizable and at least substantially water soluble macromers, having a variety of uses *in vivo*. The macromers include at least one water soluble region, at least one region which is biodegradable, usually by hydrolysis, and at least two free radical-polymerizable regions. The regions can, in some embodiments, be both water soluble and biodegradable. The macromers are polymerized by exposure of the polymerizable regions to free radicals generated, for example, by photosensitive chemicals and dyes.

An important aspect of the macromers are that the polymerizable regions are separated by at least one degradable region to facilitate uniform degradation *in vivo*. There are several variations of these polymers. For example, the polymerizable regions can be attached directly to degradable extensions or indirectly via water soluble nondegradable sections so long as the polymerizable regions are separated by a degradable section. For example, if the macromer contains a simple water soluble region coupled to a degradable region, one polymerizable region may be attached to the water soluble region and the other attached to the degradable extension or region. In another embodiment, the water soluble region forms the central core of the macromer and has at least two degradable regions attached to the core. At least two polymerizable regions are attached to the degradable regions so that, upon degradation, the polymerizable regions, particularly in the polymerized gel form, are

separated. Conversely, if the central core of the macromer is formed by a degradable region, at least two water soluble regions can be attached to the core and polymerizable regions attached to each water soluble region. The net result will be the same after gel formation and exposure to *in vivo* degradation conditions. In still another embodiment, the macromer has a water soluble backbone region and a degradable region affixed to the macromer backbone. At least two polymerizable regions are attached to the degradable regions, so that they are separated upon degradation, resulting in gel product dissolution. In a further embodiment, the macromer backbone is formed of a nondegradable backbone having water soluble regions as branches or grafts attached to the degradable backbone. Two or more polymerizable regions are attached to the water soluble branches or grafts. In another variation, the backbone may be star shaped, which may include a water soluble region, a biodegradable region or a water soluble region which is also biodegradable. In this general embodiment, the star region contains either water soluble or biodegradable branches or grafts with polymerizable regions attached thereto. Again, the polymerizable regions must be separated at some point by a degradable region.

Examples of these macromers are PEG-oligoglycolyl-acrylates. The choice of appropriate end caps permits rapid polymerization and gelation; acrylates were selected because they can be polymerized using several initiating systems, e.g., an eosin dye, by brief exposure to ultraviolet or visible light. The poly(ethyleneglycol) or PEG central structural unit (core) was selected on the basis of its high hydrophilicity and water solubility, accompanied by excellent biocompatibility. A short oligo or poly(α-hydroxy acid), such as polyglycolic

acid, was selected as a preferred chain extension because it rapidly degrades by hydrolysis of the ester linkage into glycolic acid, a harmless metabolite. Although highly crystalline polyglycolic acid is insoluble in water and most common organic solvents, the entire macromer is water-soluble and can be rapidly gelled into a biodegradable network while in contact with aqueous tissue fluids. Such networks can be used to entrap and homogeneously disperse water-soluble drugs and enzymes and to deliver them at a controlled rate. Further, they may be used to entrap particulate suspensions of water-insoluble drugs. Other preferred chain extensions are polylactic acid, polycaprolactone, polyorthoesters, and polyanhydrides. Polypeptides may also be used. Such "polymeric" blocks should be understood to include timeric, trimeric, and oligomeric blocks.

These materials are particularly useful for controlled drug delivery, especially of hydrophilic materials, since the water soluble regions of the polymer enable access of water to the materials entrapped within the polymer. Moreover, it is possible to polymerize the macromer containing the material to be entrapped without exposing the material to organic solvents. Release may occur by diffusion of the material from the polymer prior to degradation and/or by diffusion of the material from the polymer as it degrades, depending upon the characteristic pore sizes within the polymer, which is controlled by the molecular weight between crosslinks and the crosslink density. Deactivation of the entrapped material is reduced due to the immobilizing and protective effect of the gel and catastrophic burst effects associated with other controlled-release systems are avoided. When the entrapped material is an enzyme, the enzyme can be exposed to substrate while the enzyme is entrapped, provided the gel proportions are chosen to

allow the substrate to permeate the gel. Degradation of the polymer facilitates eventual controlled release of free macromolecules *in vivo* by gradual hydrolysis of the terminal ester linkages.

An advantage of these macromers are that they can be polymerized rapidly in an aqueous surrounding. Precisely conforming, semi-permeable, biodegradable films or membranes can thus be formed on tissue *in situ* to serve as biodegradable barriers, as carriers for living cells or other biologically active materials, and as surgical adhesives. In a particularly preferred embodiment, the macromers are applied to tissue having bound thereto an initiator, and polymerized to form ultrathin coatings. This is especially useful in forming coatings on the inside of tissue lumens such as blood vessels where there is a concern regarding restenosis, and in forming tissue barriers during surgery which thereby prevent adhesions from forming.

Examples demonstrate the use of these macromers and polymers for the prevention of postoperative surgical adhesions in rat cecum and rabbit uterine horn models. The polymer shows excellent biocompatibility, as seen by a minimal fibrous overgrowth on implanted samples. Hydrogels for the models were gelled in situ from water-soluble precursors by brief exposure to long wavelength ultraviolet (LWUV) light, resulting in formation of an interpenetrating network of the hydrogel with the protein and glycosaminoglycan components of the tissue. The degradable hydrogel was very effective, both by itself and in combination with tPA, in preventing adhesions.

Brief Description of the Drawings

Figure 1 shows schematically illustrated macromers of the present invention where _____ is a

water soluble core such as PEG; ~~~~~ is a hydrolyzably degradable extension such as a polyglycolide; ====== is a polymerizable end cap or side chain such as an acrylate; and ------ is a water-soluble and hydrolyzable portion such as a hyaluronate.

Figure 1A shows the degree of photopolymerization (dp) calculated and found by NMR.

Figure 2A shows Human foreskin fibroblasts cultured for six hours on glass coverslips coated with PEG 18.5K-glycolide diacrylate (18.5KG).

Figure 2B shows Human foreskin fibroblasts cultured for six hours on glass coverslips not coated with PEG.

Figure 3A shows the release of BSA from a PEG 1K (1000 molecular weight PEG) glycolide diacrylate with glycolide extensions (1 KG) hydrogel into PBS.

Figure 3B shows release of lysozyme from PEG 18.5K-DL-lactide tretraacrylate (18.5KL) into PBS.

Figure 4A shows release of active recombinant tPA from a PEG 1K lactide diacrylate (1KL) hydrogel.

Figure 4B shows release of active recombinant t-PA from PEG 4K glycolide diacrylate (4KG) hydrogel.

Figure 4C shows release of active recombinant tPA from a PEG 18.5K-glycolide diacrylate (18.5KG) hydrogel into PBS.

Figure 5A is a superior view of rabbit uterine horn used as a control. Distorted horn anatomy with 66% adhesions is evident. The horns are folded upon themselves.

Figure 5B is a superior view of rabbit uterine horn treated with a photopolymerized biodegradable hydrogel, PEG 18.5KL. Horn anatomy is normal, with no adhesion bands visible.

Figure 6A is an environmental scanning electron micrograph (ESEM) of an untreated blood vessel following trauma.

Figure 6B is an ESEM of a polymer coated blood vessel following trauma.

Description of the Preferred Embodiments

Disclosed herein are water soluble, biodegradable polymers formed from macromers containing both water soluble regions as well as biodegradable regions and at least two regions which are polymerizable by free radical initiation, preferably by photopolymerization using visible or long wavelength ultraviolet radiation.

The macromers.

In general terms, the macromers are polymers that are soluble in aqueous solutions, or nearly aqueous solutions, such as water with added dimethylsulfoxide. They have three components including a biodegradable region, preferably hydrolyzable under *in vivo* conditions, a water soluble region, and at least two polymerizable regions. Examples of these structures are shown in Figure 1.

Structure A in Figure 1 shows a macromer having a water soluble region (_____), a water soluble and degradable component (------) appended to one another. Each has a polymerizable end cap (======). Structure B shows a major water soluble component or core region (_____) extended at either end by a degradable or hydrolyzable component (~~~~~~) and terminated by, at either end, a polymerizable component (======). Structure C shows a central degradable or hydrolyzable component (~~~~~~) bound to a water soluble component (_____) capped at either end by a polymerizable component (======). Structure D shows a central water soluble component (_____) with numerous branches of hydrolyzable components (~~~~~~), each hydrolyzable component being capped with a polymerizable component (======). Structure E shows a central biodegradable, hydrolyzable component (~~~~~~) with three water

soluble branches (_____), each water soluble branch being capped by a polymerizable component (======). Structure F shows a long central water soluble and hydrolyzable component (------), each end being capped by a polymerizable component (======). Structure G shows a central water soluble and hydrolyzable component (------) capped at both ends by a hydrolyzable component (~~~~~~), each hydrolyzable component being capped by a polymerizable component (======). Structure H shows a central water soluble and degradable or hydrolyzable component (------) with end caps or branches of a polymerizable component (======). Structure I shows a central water soluble component (_____) in circular form with water soluble branches extended by a hydrolyzable component (~~~~~~) capped by a polymerizable component (======). Lastly, Structure J in Figure 1 shows a circular water soluble core component (_____) with degradable branches (~~~~~~), each being capped by a polymerizable component (~~~~~~).

The various structures shown in Figure 1 are exemplary only. Those skilled in the art will understand many other possible combinations which could be utilized for the purposes of the present invention.

Used herein is the term "at least substantially water soluble." This is indicative that the solubility should be at least about 1 g/100 ml of aqueous solution or in aqueous solution containing small amounts of organic solvent, such as dimethylsulfoxide. By the term "polymerizable" is meant that the regions have the capacity to form additional covalent bonds resulting in macromer interlinking, for example, carbon-carbon double bonds of acrylate-type molecules. Such polymerization is characteristically initiated by free-radical formation, for example, resulting from photon

absorption of certain dyes and chemical compounds to ultimately produce free-radicals.

In a preferred embodiment, a hydrogel begins with a biodegradable, polymerizable, macromer including a core, an extension on each end of the core, and an end cap on each extension. The core is a hydrophilic polymer or oligomer; each extension is a biodegradable polymer or oligomer; and each end cap is an oligomer, dimer or monomer capable of cross-linking the macromers. In a particularly preferred embodiment, the core includes hydrophilic poly(ethylene glycol) oligomers of molecular weight between about 400 and 30,000 Da; each extension includes biodegradable poly (α-hydroxy acid) oligomers of molecular weight between about 200 and 1200 Da; and each end cap includes an acrylate-type monomer or oligomer (i.e., containing carbon-carbon double bonds) of molecular weight between about 50 and 200 Da which are capable of cross-linking and polymerization between copolymers. More specifically, a preferred embodiment incorporates a core consisting of poly(ethylene glycol) oligomers of molecular weight between about 8,000 and 10,000 Da; extensions consisting of poly(lactic acid) oligomers of molecular weight about 250 Da; and end caps consisting acrylate moieties of about 100 Da molecular weight.

Those skilled in the art will recognize that oligomers of the core, extensions and end caps may have uniform compositions or may be combinations of relatively short chains or individual species which confer specifically desired properties on the final hydrogel while retaining the specified overall characteristics of each section of the macromer. The lengths of oligomers referred to herein may vary from two mers to many, the term being used to distinguish subsections or components of the macromer from the complete entity.

Water soluble regions.

In preferred embodiments, the core water soluble region can consist of poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethyloxazoline), poly(ethylene oxide)-co-poly(propyleneoxide) block copolymers, polysaccharides or carbohydrates such as hyaluronic acid, dextran, heparan sulfate, chondroitin sulfate, heparin, or alginate, proteins such as gelatin, collagen, albumin, ovalbumin, or polyamino acids.

Biodegradable regions.

The biodegradable region is preferably hydrolyzable under *in vivo* conditions. For example, hydrolyzable group may be polymers and oligomers of glycolide, lactide, ϵ-caprolactone, other hydroxy acids, and other biologically degradable polymers that yield materials that are non-toxic or present as normal metabolites in the body. Preferred poly(α-hydroxy acid)s are poly(glycolic acid), poly(DL-lactic acid) and poly(L-lactic acid). Other useful materials include poly(amino acids), poly(anhydrides), poly(orthoesters), poly(phosphazines) and poly(phosphoesters). Polylactones such as poly(ϵ-caprolactone), poly(ϵ-caprolactone), poly(δ-valerolactone) and poly(gamma-butyrolactone), for example, are also useful. The biodegradable regions may have a degree of polymerization ranging from one up to values that would yield a product that was not substantially water soluble. Thus, monomeric, dimeric, trimeric, oligomeric, and polymeric regions may be used.

Biodegradable regions can be constructed from polymers or monomers using linkages susceptible to biodegradation, such as ester, peptide, anhydride, orthoester, phosphazine and phosphoester bonds.

Polymerizable regions.

The polymerizable regions are preferably polymerizable by photoinitiation by free radical generation, most preferably in the visible or long wavelength ultraviolet radiation. The preferred polymerizable regions are acrylates, diacrylates, oligoacrylates, methacrylates, dimethacrylates, oligomethoacrylates, or other biologically acceptable photopolymerizable groups.

Other initiation chemistries may be used besides photoinitiation. These include, for example, water and amine initiation schemes with isocyanate or isothiocyanate containing macromers used as the polymerizable regions.

Photoinitiators and/or Catalysts.

Useful photoinitiators are those which can be used to initiate by free radical generation polymerization of the macromers without cytotoxicity and within a short time frame, minutes at most and most preferably seconds. Preferred dyes as initiators of choice for LWUV or visible light initiation are ethyl eosin, 2,2-dimethoxy-2-phenyl acetophenone, other acetophenone derivatives, and camphorquinone. In all cases, crosslinking and polymerization are initiated among macromers by a light-activated free-radical polymerization initiator such as 2,2-dimethoxy-2-phenylacetophenone or a combination of ethyl eosin (10^{-4} to 10^{-2} M) and triethanol amine (0.001 to 0.1 M), for example.

The choice of the photoinitiator is largely dependent on the photopolymerizable regions. For example, when the macromer includes at least one carbon-carbon double bond, light absorption by the dye causes the dye to assume a triplet state, the triplet state subsequently reacting with the amine to form a free radical which initiates polymerization. Preferred dyes for use with these materials include

eosin dye and initiators such as 2,2-dimethyl-2-phenylacetophenone, 2-methoxy-2-phenylacetophenone, and camphorquinone. Using such initiators, copolymers may be polymerized *in situ* by long wavelength ultraviolet light or by laser light of about 514 nm, for example.

Initiation of polymerization is accomplished by irradiation with light at a wavelength of between about 200-700 nm, most preferably in the long wavelength ultraviolet range or visible range, 320 nm or higher, most preferably about 514 nm or 365 nm.

There are several photooxidizable and photoreducible dyes that may be used to initiate polymerization. These include acridine dyes, for example, acriblarine; thiazine dyes, for example, thionine; xanthine dyes, for example, rose bengal; and phenazine dyes, for example, methylene blue. These are used with cocatalysts such as amines, for example, triethanolamine; sulphur compounds, for example, RSO_2R^1; heterocycles, for example, imidazole; enolates; organometallics; and other compounds, such as N-phenyl glycine. Other initiators include camphorquinones and acetophenone derivatives.

Thermal polymerization initiator systems may also be used. Such systems that are unstable at 37°C and would initiate free radical polymerization at physiological temperatures include, for example, potassium persulfate, with or without tetraamethyl ethylenediamine; benzoylperoxide, with or without triethanolamine; and ammonium persulfate with sodium bisulfite.

Applications for the Macromers.

Prevention of Surgical Adhesions.

A preferred application is a method of reducing formation of adhesions after a surgical procedure in a patient. The method includes coating damaged tissue surfaces in a patient with an aqueous solution of a

light-sensitive free-radical polymerization initiator and a macromer solution as described above. The coated tissue surfaces are exposed to light sufficient to polymerize the macromer. The light-sensitive free-radical polymerization initiator may be a single compound (e.g., 2,2-dimethoxy-2-phenyl acetophenone) or a combination of a dye and a cocatalyst (e.g., ethyl eosin and triethanol amine).

Controlled drug delivery.

A second preferred application concerns a method of locally applying a biologically active substance to tissue surfaces of a patient. The method includes the steps of mixing a biologically active substance with an aqueous solution including a light-sensitive free-radical polymerization initiator and a macromer as described above to form a coating mixture. Tissue surfaces are coated with the coating mixture and exposed to light sufficient to polymerize the macromer. The biologically active substance can be any of a variety of materials, including proteins, carbohydrates, nucleic acids, and inorganic and organic biologically active molecules. Specific examples include enzymes, antibiotics, antineoplastic agents, local anesthetics, hormones, antiangiogenic agents, antibodies, neurotransmitters, psychoactive drugs, drugs affecting reproductive organs, and oligonucleotides such as antisense oligonucleotides.

In a variation of the method for controlled drug delivery, the macromers are polymerized with the biologically active materials to form microspheres or nanoparticles containing the biologically active material. The macromer, photoinitiator, and agent to be encapsulated are mixed in an aqueous mixture. Particles of the mixture are formed using standard techniques, for example, by mixing in oil to form an emulsion, forming droplets in oil using a nozzle, or forming droplets in air using a nozzle. The

suspension or droplets are irradiated with a light suitable for photopolymerization of the macromer.

Tissue Adhesives.

Another use of the polymers is in a method for adhering tissue surfaces in a patient. The macromer is mixed with a photoinitiator or photoinitiator/cocatalyst mixture to form an aqueous mixture and the mixture is applied to a tissue surface to which tissue adhesion is desired. The tissue surface is contacted with the tissue with which adhesion is desired, forming a tissue junction. The tissue junction is then irradiated until the macromers are polymerized.

Tissue Coatings.

In a particularly preferred application of these macromers, an ultrathin coating is applied to the surface of a tissue, most preferably the lumen of a tissue such as a blood vessel. One use of such a coating is in the treatment or prevention of restenosis, abrupt reclosure, or vasospasm after vascular intervention. The photoinitiator is applied to the surface of the tissue, allowed to react, adsorb or bond to tissue, the unbound photoinitiator is removed by dilution or rinsing, and the macromer solution is applied and polymerized. As demonstrated below, this method is capable of creating uniform polymeric coating of between one and 500 microns in thickness, most preferably about twenty microns, which does not evoke thrombosis or localized inflammation.

Tissue Supports.

The macromers can also be used to create tissue supports by forming shaped articles within the body to serve a mechanical function. Such supports include, for example, sealants for bleeding organs, sealants for bone defects and space-fillers for vascular aneurisms. Further, such supports include strictures

to hold organs, vessels or tubes in a particular position for a controlled period of time.

The following examples are presented to describe preferred embodiments and utilities of the present invention and are not meant to limit the invention unless otherwise stated in the claims appended hereto. Taken together, the examples illustrate representative demonstrations of the best mode of implementing the invention as currently understood.

Table 1 shows the code names of the various macromers synthesized in or for use in the examples, along with their composition in terms of the molecular weight of the central PEG segment and the degree of polymerization of the degradable comonomer.

Table 1: Macromer Molecular Weight and Composition.

PEG molecular weight	Comonomer	D.P. of conomoner per OH group	Polymer Code
20,000	glycolide	15	20KG
18,500	glycolide	2.5	18.5K
10,000	glycolide	7	10KG
6,000	glycolide	5	6KG
4,000	glycolide	5	4KG
1,000	glycolide	2	1KG
20,000	DL-lactide	10	20KL
18,500	DL-lactide	10	18.5KL
10,000	DL-lactide	5	10KL
6,000	DL-lactide	5	6KL
1,000	DL-lactide	2	1KL
600	DL-lactide	2	0.6KL
600	DL-lactide + caprolactone(CL)	lactide 2; CL 1	0.6KLCL
18,500	caprolactone	2.5	18.5KCL
18,500	-	-	18.5KCO

Example 1: Synthesis of Photopolymerized Biodegradable Hydrogels.

PEG-based hydrogels

PEG-based biodegradable hydrogels are formed by the rapid laser or UV photopolymerization of water soluble macromers. Macromers, in turn, are synthesized by adding glycolic acid oligomers to the end groups of PEG and then capping with acrylic end groups. The PEG portions of the macromers confer water solubility properties, and subsequent polymerization results in cell-nonadhesive hydrogels. Glycolic acid oligomers serve as the hydrolyzable fraction of the polymer network, while acrylic end groups facilitate rapid polymerization and gelation of the macromers.

In preparation for synthesis, glycolide (DuPont) or DL-lactide (Aldrich) was freshly recrystallized from ethyl acetate. PEG oligomers of various molecular weight (Fluka or Polysciences) were dried under vacuum at 110°C prior to use. Acryloyl chloride (Aldrich) was used as received. All other chemicals were of reagent grade and used without further purification.

Macromer synthesis

A 250 ml round bottom flask was flame dried under repeated cycles of vacuum and dry argon. 20 gm of PEG (molecular weight 10,000), 150 ml of xylene and 10 μgm of stannous octoate were charged into the flask. The flask was heated to 60°C under argon to dissolve the PEG and cooled to room temperature. 1.16 gm of glycolide was added to the flask and the reaction mixture was refluxed for 16 hr. The copolymer was separated on cooling and was recovered by filtration. This copolymer was separated on cooling and recovered by filtration. This copolymer (10K PEG-glycolide) was used directly for subsequent reactions. Other polymers were similarly synthesized

using DL-lactide or ϵ-caprolactone in place of glycolide and using PEG of different molecular weights.

Synthesis of photosensitive oligomers (macromers):

19 gm of 10K PEG-glycolide copolymer was dissolved in 150 ml methylene chloride and refluxed with 1 ml acryloyl chloride and 1.2 ml of triethylamine for 12 hr under an argon atmosphere. The solid triethylamine hydrochloride was separated by filtration and the polymer was precipitated by adding the filtrate to a large excess of hexane. The polymer (capped by an acrylate at both ends) was further purified by repeated dissolution and precipitation in methylene chloride and hexane respectively.

Table 2 lists certain macromers synthesized. The degree of polymerization of the glycolide chain extender was kept low so that all polymers have approximately 10 ester groups per chain, or about 5 per chain end. When these polymers are photopolymerized, a crosslinked three-dimensional network is obtained. However, each chain segment in the resulting network needs just one ester bond cleaved at either end to "degrade." These ester cleavages enable the chain to dissolve in the surrounding physiological fluid and thereby be removed from the implant site. The resulting hydrolysis products, PEG and glycolic acid, are water soluble and have very low toxicity.

TABLE 2: Macromers Synthesized

Polymer Code	Mol. Wt. of Central PEG Chain (daltons)	%Glycolide in Extremeties	% ε-Caprolactone in Extremities	Calculated Mol. Wt. of Extremities (daltons)	Appearance
0.4K	400	100	-	580	Viscous liquid
1KG	1000	100	-	300	Viscous liquid
4KG	4000	100	-	232	White solid
10KG	10000	100	-	580	White solid
18.5KG	18500	100	-	1160	Yellow solid
col8.5KGCL	18500	50	-	580	White solid

Due to the presence of only a few units of glycolic acid per oligomeric chain, the solubility properties of the photocrosslinkable prepolymers are principally determined by the central PEG chain. Solubility of the macromers in water and methylene chloride, both of which are solvents for PEG, is not adversely affected as long as the central PEG segment has a molecular weight of 1,000 daltons or more. Solubility data for the prepolymers synthesized is given in Table 3.

Table 3: SOLUBILITY DATA

Solvent	1KG	4KG	10KG	18.5KG	TMP*
DMSO	–	■	–	■	■
Acetone	–	■	■	■	–
Methanol	–	■	–	■	–
Water	–	–	–	–	■
Hexane	■	■	■	■	■
Methylene Chloride	–	–	–	–	–
Cold Xylene	■	■	■	■	–
Hot Xylene	–	–	–	–	–
Benzene	■	■	■	■	–

– Soluble

■ Not Soluble

* Trimethylolpropane glycolide triacrylate

PEG chains with different degrees of polymerization of DL-lactide were synthesized to determine the degree of substitution for which water solubility of the macromers can be retained. The results are shown in Table 4. Beyond about 20% substitution of the hydrophilic PEG chain with hydrophobic DL-lactoyl or acrylate terminals leads to the macromers becoming insoluble in water, though they

are still soluble in organic solvents such as methylene chloride.

Table 4: Solubility of Macromers

D.P.* of Ethylene Oxide	D.P.* of lactide or glycolide	% extension of PEG chain	Solubility in water
420	4	0.1	soluble
420	10	2.4	soluble
420	20	4.8	soluble
420	40	9.5	soluble
420	80	19	insoluble
23	2	8.7	soluble
23	4	17.4	soluble
23	10	43.5	insoluble
23	40	174	insoluble
5	4	80	insoluble
10	4	40	soluble

* degree of polymerization

Photopolymerization

The macromers can be gelled by photopolymerization using free radical initiators, with the presence of two acrylic double bonds per chain leading to rapid gelation. A 23% w/w solution of various degradable polymers in HEPES buffered saline containing 3 μl of initiator solution (300 mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone in n-vinyl pyrrolidone) was used. 100 μl of the solution was placed on a glass coverslip and irradiated with a low intensity long wavelength UV (LWUV) lamp (Blak-Ray, model 3-100A with flood). The times required for gelation to occur were noted and are given below. These times are typically in the range of 10 seconds. This is very significant because these reactions are carried out in air (UV initiated photopolymerizations are slow in air as compared to an inert atmosphere)

and using a portable, low powered long wave UV (LWUV) emitting source. Oxygen, which often inhibits free radical reactions by forming species which inhibit propagation, did not seem to slow down the polymerization. Such fast polymerizations are particularly useful in applications requiring *in situ* gelations. This rapid gelation is believed to be due to the formation of micelle-like structures between the relatively hydrophobic polymerizable groups on the macromer, thereby increasing the local concentration of the polymerizable species in aqueous solution and increasing polymerization rates.

Visible laser light is also useful for polymerization. Low intensity and short exposure times make visible laser light virtually harmless to living cells since the radiation is not strongly absorbed in the absence of the proper chromophore. Laser light can also be transported using fiber optics and can be focused to a very small area. Such light can be used for rapid polymerization in highly localized regions; gelation times for selected prepolymers are given in Table 5. In each case, 0.2 ml of a 23% w/v photosensitive oligomer solution is mixed with ethyl eosin (10^{-4} M) and triethanol amine (0.01 to 0.1 M) and the solution is irradiated with an argon ion laser (American argon ion laser model 905 emitting at 514 nm) at a power of 0.2-0.5 W/cm^2. The beam is expanded to a diameter of 3 mm and the sample is slowly scanned until gelation occurs.

Table 5: Gelation Times

Polymer	UV polymerization * gelation time (mean ±S.D.) (s)	Laser Polymerization** gelation time (s)
1KG	5.3±4.1	<1
4KG	14.7±0.5	<1
6KG	9.3±0.5	<1
10KG	18.±0.8	<1
10KL	7.7±0.5	<1
18KG	23.3±1.2	<1
20KG	13.3±0.5	<1

* Initiator: 2,2-dimethoxy-2-phenylacetophenone, concentration 900 ppm: 0.2 ml of 23% monomer solution in PBS

** Argon ion laser emitting at 514nm. power 3 W/cm^2: ethyloeosin, triethanol amine initiating system: 0.2 ml of 23% monomer solution in PBS

Biodegradability

Biodegradation of the resulting polymer network is an important criteria in many biomedical applications. Degradation of poly(glycolic acid and poly(DL-lactic acid) has been well documented in the literature. The degradation mainly takes place through the hydrolysis of the ester bond; the reaction is second order and highly pH dependent. The rate constant at pH 10 is 7 times faster than that at pH 7.2.

Such facile biodegradation is surprising because poly(α-hydroxyacidesters) are hydrophobic and highly insoluble in water. Accessibility of the polymer matrix to the aqueous surrounding is therefore limited. However, because the networks are hydrogels which are swollen with water, all the ester linkages in the network are in constant contact with water with the aqueous surroundings. This results in a uniform

bulk degradation rather than a surface degradation of these gels.

Table 6 gives hydrolysis data for some of these networks; times listed are for complete dissolution of 60 mg of gel at pH 7.2 and 9.6. As noted, most of the gels dissolve within 12 hours at pH 9.6. 18.5k gel dissolves within 2.5 hr at pH 9.6 whereas 18.5KCO gel does not dissolve in 3 days, indicating that the lactoyl, glycoloyl, or ϵ-caprolactoyl ester moiety is responsible for degradation of these networks. It also can be seen that the 18.5KG gel hydrolyzes more rapidly than the 4KG gel. This may be due to the reduced hydrophilicity and higher crosslink density of the latter gel.

Table 6: Hydrolysis Data

Oligomer used for gelation	Time taken to dissolve gel at pH 9.6 (h)	Time taken to dissolve gel at pH 7.2 (days)
4KG	6.2	5.5
10KG	12.25	5.5
18.5KG	2.25	>7
18.5KCL	>5 days	>7
18.5KCO	>5 days	>7

Characterization of macromers

FTIR spectra of the prepolymers were recorded on a DIGILAB model FTS 15/90. The absorption at 1110 cm^{-1} (characteristic C-O-C absorption of PEG) shows the presence of PEG segments. The strong 1760 cm^{-1} absorption shows the presence of glycolic ester. The absence of hydroxyl group absorption around 3400 cm^{-1} and a weak acrylic double bond absorption at 1590 cm^{-1} shows the presence of acrylic double bonds at the end groups.

500 MHz proton and 125 MHz carbon-13 spectra were recorded on a GE 500 instrument. The presence of a very strong peak at 4.9 ppm due to CH_2 methylene from the PEG segment, a peak at 5.09 ppm due to the

glycolic ester segment and an acrylic proton singlet at 5.8 ppm can be easily seen from proton NMR. The estimated molecular weight of PEG segment and glycolic acid segment for different copolymers is shown in Table 2. The carbonyl peak at 169.39 ppm from glycolic acid and 36.5 ppm peak from methylene carbons from PEG in carbon-13 NMR are consistent with the reported chemical composition of these copolymers.

Differential scanning calorimetry (Perkin Elmer DSC-7) was used to characterize the oligomers for thermal transitions. The oligomers were heated from -40°C to 200°C at a rate of 20°C/min, presumably causing polymerization. The polymer was then cooled to -40°C at a rate of 60°C/min and again heated to 200°C at a rate of 20°C/min. The first scans of biodegradable 18.5K PEG glycolide tetraacrylate (18.5KG) oligomer were compared to that of the non-degradable 18.5K PEG tetraacrylate (18.5KCO) scan. It was seen that a glass transition appears in the 18.5KG at -2°C while no such transition exists in the 18.5KCO. A small melting peak at 140°C was also evident due to the few glycolic acid mers which can crystallize to a limited extent. The melting peak for PEG is shifted downwards in 18.5KG to 57°C from 60.7°C for 18.5KCO. This is probably due to disturbance of the PEO crystalline structure due to the presence of the glycolic acid linkages. In the third cycle, by which time the oligomers have presumably polymerized, the Tg and Tm transitions for the glycolide segments can no longer be seen, indicating that a crosslinked network has formed and the glycolic acid segments are no longer capable of mobility.

The degree of polymerization (D.P.) of the degradable segments added to the central water soluble PEG chain was determined in several cases using ^1H NMR. The experimentally determined D.P. was seen to be in good agreement with the calculated number, as shown by

Figure 1A. Thus, the ring opening reaction initiated by the PEG hydroxyls proceeds to completion, giving quantitative yields.

Determination of Total Water, Free Water Bound Water

Solutions of various degradable macromers were made as described above. Gels in the shape of discs were made using a mold. 400 μl of solution was used for each disc. The solutions were irradiated for 2 minutes to ensure thorough gelation. The disc shaped gels were removed and dried under vacuum at 60°C for 2 days. The discs were weighed (W1) and then extracted repeatedly with chloroform for 1 day. The discs were dried again and weighed (W2). The gel fraction was calculated as W2/W1. This data appears in Table 7.

Subsequent to extraction, the discs were allowed to equilibrate with PBS for 6 hours and weighed (W3 after excess water had been carefully swabbed away). The total water content was calculated as (W3-W2) X 100/W3. Differential scanning calorimetry (DSC) was used to determine the amount of free water that was available in the gels. A scan rate of 20°C/min was used and the heat capacity for the endotherm for water melting was measured (H1). The heat capacity of HBS was also measured (H2). The fraction of free water was calculated as H1/H2. The residual water was assumed to be bound due to hydrogen bonding with the PEO segments. The presence of free water in the gels was indicated. This free water can be expected to help proteins and enzymes entrapped in such gels in maintaining their native conformation and reducing deactivation. Thus these gels would appear to be suited for controlled release of biological micromolecules. The data for gel water content is summarized in Table 7

Table 7: Hydrogel Water content

Polymer Code	% Free Water	% Bound Water	% Total Water	% Gel Content
1KG	68.4	14	82.3±2.6	61.3±5.2
4KG	78.0	9.3	87.3±1.8	56.3±0.9
6KG	74.8	13.4	88.1±3.3	66.5±2.35
10KG	83.7	10.8	94.5±0.5	54.3±0.6
10KL	82.0	9.7	91.7±0.5	63.9±3.7
18.5KG	71.8	22.3	94.0±0.4	47.0±4.9
20KG	79.8	14.8	94.5±0.4	44.5±4.8

Example 2: Use of multifunctional macromers.

30 g of a tetrafunctional water soluble PEG (MW 18,500) (PEG 18.5k) was dried by dissolving the polymer in benzene and distilling off the water benzene azeotrope. In a glove bag, 20 g of PEG 18.5 k, 1.881 g of glycolide and 15 mg of stannous octoate were charged into a 100 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 200 ml of dichloromethane in a 500 ml round bottom flask cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 h. at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day.

Example 3: Synthesis of a photosensitive macromer containing DL-lactide.

PEG (MW) 20,000) (PEG 20k) was dried by dissolving in benzene and distilling off the water benzene azeotrope. In a glove bag, 32.43 g of PEG 20k, 2.335 g of DL-lactide and 15 mg of stannous octoate were charged into a 100 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 200 ml of dichloromethane in a 500 ml round bottom flask cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 hours at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day.

Example 4: Synthesis of a Photosensitive Precursor Containing DL-Lactide and ϵ-Caprolactone.

PEG (MW 600) (PEG 0.6k) was dried by dissolving in benzene and distilling off the water benzene azeotrope. In a glove bag, 0.973 g of PEG 0.6k, 0.467 g of DL-lactide along with 0.185 g of ϵ-caprolactone and 15 mg of stannous octoate were charged into a 50 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by

pouring into an excess of dry ethyl ether. It was redissolved in 50 ml of dichloromethane in a 250 ml round bottom flask cooled to 0°C. to this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 hours at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day and was a liquid at room temperature.

Example 5: Selection of dyes for use in photopolymerization.

It is possible to initiate photopolymerization with a wide variety of dyes as initiators and a number of electron donors as effective cocatalysts. Table 8 illustrates photopolymerization initiated by several other dyes which have chromophores absorbing at widely different wavelengths. All gelations were carried out using a 23% w/w solution of 18.5KG in HEPES buffered saline. These initiating systems compare favorably with conventional thermal initiating systems, as can also be seen from Table 8. Other photoinitiators that may be particularly useful are 2-methoxy-2-phenyl acetophenone and camphorquinone.

Table 8: Polymerization Initiation of 18.5KG PEG

INITIATOR	LIGHT SOURCE*	TEMPERATURE °C	GEL TIME (SEC)
Eosin Y, 0.00015M; Triethanolamine 0.65M	S1 with UV filter	25	10
Eosin Y, 0.00015M; Triethanolamine 0.65M	S4	25	0.1
Methylene Blue, 0.00024M; p-toluenesulfinic acid, 0.0048M	S3	25	120
2,2-dimethoxy-2-phenyl acetophenone 900 ppm	S2	25	8
Potassium persulfate 0.0168M	-	75	180
Potassium Persulfate 0.0168M; tetramethyl ethylene-diamine 0.039M	-	25	120
Tetramethyl ethylene-diamine 0.039M; Riboflavin 0.00047M	S1 with UV filter	25	300

*LIST OF LIGHT SOURCES USED

CODE	SOURCE
S1	Mercury lamp, LEITZ WETSLER Type 307-148.002, 100W
S2	Black Ray longwave UV lamp, model B-100A W/FLOOD
S3	MELLES GRIOT He-Ne laser, 10mW output, 1=632 nm
S4	American laser corporation, argon ion laser, model 909BP-15-01001; λ=488 and 514 nm

Numerous other dyes can be used for photopolymerization. These dyes include but are not limited to: Erythrosin, phloxine, rose bengal, thioneine, camphorquinone, ethyl eosin, eosin, methylene blue, and riboflavin. The several possible cocatalysts that can be used include but are not limited to: N-methyl diethanolamine, N,N-dimethyl benzylamine, triethanol amine, triethylamine, dibenzyl

amine, N-benzyl ethanolamine, N-isopropyl benzylamine, and N-vinyl pyrrolidinone.

Example 6: Thermosensitive Biodegradable Gels from N-Isopropyl Acrylamide.

Synthesis of low molecular weight polyisopropyl acrylamide.

N-isopropyl acrylamide (NIPAAm) was recrystallized from 65:35 hexane benzene mixture. Azobisisobutyronitrile (AIBN) was recrystallized from methanol. 1.5 g of NIPAAm was polymerized using 3 mg of AIBN and 150 mg of mercaptoethanol in 1:1 acetone water mixture (24 hours at 65°C). The viscous liquid after polymerization was purified by dissolving in acetone and precipitating in diethyl ether. Yield 80%.

This hydroxy terminated low molecular weight poly(NIPAAm) was used in chain extension reactions using glycolide and subsequent endcapping reaction using acryloyl chloride as described in other examples.

1 g of modified poly(NIPAAm) based oligomer and 0.2 g 1KL were dissolved in water at 0°C and polymerized at 0°C using 2-2-dimethoxy-2-phenylacetophenone (900 PPM).

Example 7: *In Vitro* Degradation

The gels were extracted as described in Example 1 to remove the unpolymerized macromer fraction fraction and the gels were then placed in 50 mM HEPES buffered saline (0.9% NaCl), pH 7.4 at 37°C. Duplicate samples were periodically removed, washed with fresh HBS and dried at 100°C for 1 day and weighed to determine mass loss in the gel. The compositions of the various gels used were the same as described in the previous examples. Table 9 shows the extent of degradation of these gels given as percent of mass lost over time. The respective times are given in parenthesis along with the mass loss data.

Tabl 9:	Gel Degradation
1KG	20.1% (1 d), 20.36±0.6 (2d), 21.7± (6d), 28.8±16.6 (10 d) estimated total Degradation time 45 days.
4KG	38.9 (1d), 60.3±4.2 (2d), 78.9 (3d), 99.3±4.7 (6d). Total degradation time 5.5 days.
6KG	18.3±6.8 (1d), 27.4±1.0 (2d), 32.8±11.3 (3d), 104.8±3.2 (5d). total degradation time 4.5 days 10KG 0.6±0.6 (8 hr), 100 (1d). Total degradation time 1 day.
10KL	10.0±4.84 (2d), 6.8±1.7 (3d), 4.5±3.1 (6d), 8.0±0.2 (10d). Total degradation time estimated to be 20 days.
20KG	68.1±4.2 (8hr), 99.7±0.3 (1d). Total degradation time 15 hr.

Example 8: Fibroblast adhesion and spreading.

The *in vitro* response of Human foreskin fibroblast (HFF) cells to photopolymerized gels was evaluated through cell culture on polymer networks. 0.2 ml of monomer solution was UV polymerized on an 18 x 18 mm glass coverslips under sterile conditions. HFF cells were seeded on these gels at a cell density of 1.8×10^4 cells/sq cm of coverslip area in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum. The gels were incubated for 6 hr at 37°C in a 5% CO_2 environment, at the end of which they were washed twice with phosphate buffered saline (PBS). The adherent cells were fixed using a 2% glutaraldehyde solution in PBS. The gels were examined under a phase contrast microscope at a magnification of 200X, and the number of adherent and spread cells evaluated by examining five fields selected at predetermined locations on the coverslips.

The number of adherent cells is reported in Table 10 along with those for glass control surfaces.

Cell adhesion is seen to be dramatically lowered on gel-coated glass.

Table 10: Cell Adhesion

Surface	Attached Cells/cm^2
glass	13220±3730
18.5KG	250±240
18.5KCL	1170±1020
18.5KCO	390±150

Typical photographs of these cells on the 18.5KCL gel surfaces and on control glass surfaces are shown in Figures 2A and 2B. It can be easily seen from Table 10 that these gels are highly resistant to cellular growth. Even the 18.5KCL is still less than 10% of the glass. Cells attached to the glass surface show a flattened and well-spread morphology whereas the few cells that are attached to the gel are rounded and loosely attached. This may result from the fact that hydrated PEG chains have a high motility and have been shown to be effective in minimizing protein adsorption. One of the mechanisms by which cell adhesion is mediated is through the interaction of cell surface receptors with adsorbed cell adhesion proteins. Thus the reduction in overall protein adsorption results in minimal cell adhesion protein adsorption and reduced cell adhesion.

Example 9: Release of Protein (Bovine Serum Albumin) from Polymers.

1KG was used for this study. This macromer was liquid at room temperature and was used as such. 1 mg of bovine serum albumin (BSA) was added per ml of monomer solution along with 0.9 mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone as initiator. The protein was dissolved in the monomer solution and disc shaped gels were made by exposing 0.2 g of macromer mixture to LWUV for 1 min. Two such discs were placed in a flask containing 20 ml of PBS and incubated at 37°C. Two aliquots of 20 μl each were removed from

these flasks periodically and the amount of BSA released was assayed using the Bio-Rad total protein assay. The release profile for BSA is shown in Figure 3A. It can be seen that the release of BSA is relatively steady over more than a month.

Example 10: Enzyme Release Assay

Water solubility of the macromers means gelation can be carried out in a non-toxic environment. This makes these materials suitable for intraoperative uses where *in situ* gelation is needed. Since the precursors are water soluble, the gels can be used as drug delivery vehicles for water soluble drugs, especially macromolecular drugs such as enzymes, which would otherwise be denatured and lose their activity. Release of lysosome and tPA from the polymers was used to illustrate the feasibility of using biodegradable hydrogels for controlled release of biomolecules.

Lysozyme release

The enzyme lysozyme (MW:14,400) is a convenient model for release of a low molecular weight protein from a biodegradable gel. The Biorad total protein assay was used to quantify the enzyme released. The enzyme was dissolved in PBS at a concentration of 20 mg/ml. The monomer PEG-dl-lactic acid-diacrylate was dissolved in PBS to produce a 40% solution. The lysozyme solution was added to the monomer solution to attain a 24% monomer solution. The monomer/lysozyme solution was polymerized under UV in a cylindrical mold, using 30 μl of the initiator 2,2-dimethoxy-2-phenyl-acetophenone in 1-vinyl-2-pyrrolidone (30 mg/ml) as the initiator. The polymer was cut into 10 equal sized pieces and immersed in 10 ml PBS. Samples of the PBS were withdrawn at intervals and assayed for lysozyme released into the PBS. Lysozyme was released from the PEG-DL-lactic acid-diacrylate gel over an 8 day interval, with the maximum rate of release

occurring within the first 2 days, as shown by Figure 3B.

Release of recombinant t-PA

Three macromers were used for these studies: 1KL, 4KG, and 18.5KG. The 1KL macromer was liquid at room temperature and was used as such. The second macromer, 4KG, was used as a 75% w/w solution in PBS. The third composition was a mixture of equal parts of 1KL and a 50% w/w solution of 18.5KG. 3.37 mg of tissue plasminogen activator (single chain, recombinant, M.W. 71,000) was added per gram of macromer solution along with 0.9 mg/ml of 2,2 dimethoxy 2 phenyl acetophenone as initiator. The protein was dissolved with the macromer and disc shaped gels were made by exposing 0.2 g of macromer mixture to LWUV for 1 minute. Two such discs were rinsed with PBS, placed in a flask containing 5 ml of PBS and incubated at 37°C. Two aliquots of 100 μl each were removed from these flasks periodically and the amount of active t-PA released was assayed using a chromogenic substrate assay (Kabi-vitrum). The release profiles from the 1K lactide gels, 4K glycolide gels, and the 50/50 1K glycolide/18.5K glycolide are shown in Figures 4A - 4C. Fully active tPA can be released for periods up to at least two months.

By selecting an appropriate formulation, the release rate can be tailored for a particular application. It is also possible to combine formulations with different molecular weights so as to synergistically achieve appropriate attributes in release and mechanical characteristics.

For prevention of postoperative adhesions, in addition to the barrier effect of the gels, the gels can be loaded with a fibrinolytic agent to lyse incipient filmy adhesions which escape the barrier

effect. This further enhances the efficacy of biodegradable gels in adhesion prevention.

Example 11: Toxicity of Polymers and Commercial Adhesives.

To evaluate the toxicity of *in situ* polymerization of the macromer solutions described herein, as compared to commercial adhesives, 100 μl of 18.5KCO prepolymer solution was placed on the right lobe of a rat liver and gelled by exposing it to LWUV for 15 sec; similarly, a few drops of a n-butyl cyanoacrylate based glue were placed on the left lobe. The liver was excised after a week, fixed in 10% neutral buffered formalin, blocked in paraffin, sectioned and stained using hematoxylin and eosin.

No adverse tissue reaction was evident on the surface of the lobe exposed to the biodegradable gel. No inflammatory reaction to the polymerization process can be seen. The epithelium looks normal, with no foreign body reaction.

In comparison, the lobe exposed to cyanoacrylate glue shows extensive tissue necrosis and scarring with 10-30 cell deep necrotic tissue. Fibrosis is evident in the necrotic portions close to underlying normal tissue.

Example 12: Prevention of Post-Surgical Adhesions with Photopolymerized Biodegradable Polymer.

A viscous sterile 23% solution in phosphate buffered saline (8.0 g/l NaCl, 0.201 g/l KCl, 0.611 g/l Na_2HPO_4, 0.191 g/l KH_2PO_4, pH 7.4) of polyethylene glycol (M.W. 18,500) which has been chain extended on both ends with a short polyglycolide repeat unit (average number of glycolidyl residues: 10 on each end) and which has been subsequently terminated with an acrylate group was prepared. Initiator needed for the crosslinking reaction, 2,2-dimethoxy-2-phenyl acetophenone, was added to the macromer solution to

achieve an initiator concentration of 900 ppm. A 30 second exposure to a long wave UV lamp (Blak Ray) is sufficient to cause polymerization.

Animal models evaluated

Animal models evaluated included a rat cecum model and a rabbit uterine horm model. In the rat cecum mode, 6 out of 7 animals treated with the macromer solution showed no adhesions whatsoever, while untreated animals showed consistent dense adhesion formation. In the rabbit uterine horn model, a significant ($p<0.01$) reduction in adhesion formation was seen in the animals treated with the gel. Studies conducted in rats using only the ungelled viscous precursor solution (no LWUV) failed to prevent the formation of adhesions.

Rat cecum model

Twenty-one Sprague Dawley male rats having an average weight of 250 gm were divided into three groups for treatment and two for controls. The abdomen was shaved and prepared with a betadine solution. A midline incision was made under Equithesin anesthesia. The cecum was located and 4 to 5 scrapes were made on a region about 2 x 1 cm on one side of the cecum, using a 4 x 4 in gauze pad to produce serosal injury and punctate bleeding. The abdominal incisions in these animals were closed using a continuous 4-0 silk suture for the musculoperitoneal layer and 7.5 mm stainless steel staples for the cutaneous layer. A topical antibiotic was applied at the incision site.

The first group consisted of 7 animals serving as controls without treatment, to confirm the validity of the model. The second group served as a control with the application of the precursor but without photopolymerization to form the hydrogel. After induction of the cecal injury, about 0.25 ml of the precursor solution was applied to the injury site

using a pipet. The abdominal incision was then closed as above.

The third group served as the gel treatment group and was prepared as the second group except that the precursor film was exposed to a LWUV lamp for 45 seconds to cause gelation. Both the obverse and reverse sides of the cecum were similarly treated with precursor and light. No attempt was made to dry the surface of the tissue, to remove blood, or to irrigate the area prior to treatment.

The animals were sacrificed at the end of two weeks by CO_2 asphyxiation. The incisions were reopened and adhesions were scored for location, extent, and tenacity. The extent of adhesions was reported as a percentage of the traumatized area of the cecum which forms adhesions with adnexal organs or the peritoneal wall. Tenacity of the adhesions was scored on a scale from 0 to 4: no adhesions - grade 0; tentative transparent adhesions which frequently separate on their own - grade 1; adhesions that give some resistance but can be separated by hand - grade 2; adhesions that require blunt instrument dissection to separate - grade 3; and dense thick adhesions which require sharp instrument dissection in the plane of the adhesion to separate -grade 4.

Rat cecum model results

The control group without treatment shows consistently dense and extensive adhesions. The extent of abraded area covered with adhesions was seen to be 73±21% (mean ± S.D., n=7). The severity of adhesions was grade 3.5±0.4. Most of the adhesions were dense and fibrous, involving the cecum with itself, with the peritoneal wall and with other organs such as the liver, small intestine, and large intestine. Frequently the nesentery was seen to be involved in adhesions. In the control group with the application of precursor solution but without gelation

by exposure to the LWUV lamp, the extent of adhesion was 60 ± 24% (n=7), and the severity of adhesions was 3.1 ± 0.4. In the gel treated group, the cecum was seen to be completely free of adhesions in 6 out of 7 animals. In one case, a grade 2 adhesion was seen with the mesentery over 10% of the area and a grade 2.5 adhesion was seen over 15% of the area, bridging the cecum to the sutures on the site of the incision in the peritoneal wall. The overall adhesion extent for the group was 4%, and the overall severity was 0.32. No evidence of residual gel was visible, the gel presumably having degraded within the prior two weeks. The cecum appeared whitish with a fibrous layer on the surface in the control group, but the tissue appeared healthy and normal in animals treated with the gel.

Rabbit uterine horn model

Eight sexually mature female New Zealand rabbits between 2 and 3 kg in weight were prepared for surgery. A midline incision was made in the lower abdominal region under Rompun, Ketamine, and Acepromazine anesthesia. The uterine horns were located and the vasculature to both horns was systematically cauterized to induce an ischemic injury. One animal was rejected from the study due to immature uterine horns. Seven rabbits were selected for the treatment with only the photopolymerizable hydrogel and two animals were selected for evaluating the combined efficacy of the hydrogel with a fibrinolytic agent, tissue plasminogen activator (tPA). 5 mg of tPA/ml macromer solution was used in the latter case. After cauterization, macromer solutions (0.5 ml) were applied along the horn and allowed to coat the surface where the cauterization injury had been induced. After uniform application of the solution was complete, the horns were exposed to a LWUV lamp for 1 min to induce gelation. The procedure

was repeated on the reverse side of the horns. The incisions were then closed using a continuous 2-0 Vicryl (Ethicon) suture for the musculoperitoneal layer and a 0 Vicryl (Ethicon) suture for the cutaneous layer. No prophylactic antibiotics were administered. No postoperative complications or infections were observed. Five animals were used in the control group. The ischemic injury was made as described and the incision was closed without the application of the precursor; all techniques were identical between the treatment group and the control group.

Controls were used where the same animal model was subjected to surgery without application of the macromer; all surgical techniques were identical between the treatment group and the historical controls.

The rabbits were reoperated under Ketamine anesthesia at the end of two weeks to evaluate adhesion formation; they were sacrificed by introcardiac KCl injection. Adhesion formation was evaluated for extent and tenacity. Extent of adhesion formation was evaluated by measuring the length of the uterine horn that formed adhesions with itself or with the peritoneal wall or other organs. Tenacity of adhesion was classified as either filmy or fibrous. Filmy adhesions were usually transparent, less strong, and could be freed by hand. The fibrous adhesions were dense, whitish, and usually required sharp instrument dissection to be freed. In cases where only a single filmy adhesion band was evident, a score of 5% was assigned.

Typical samples of the horn were excised for histology and were fixed in a 10% neutral buffered formalin solution. Paraffin sections of the samples were stained using hematoxylin and eosin.

Rabbit uterine horn model results

The adhesion score is the % of affected area occupied by the adhesions, with grading of each as being filmy or fibrous. Distorted horn anatomies were observed in control animals. The mean score in the control group was 50 ± 15% of the affected area of the horn being occupied by adhesions with 10% of these being filmy and 90% fibrous. Distorted horn anatomies were observed, as can be seen from Figure 5A which presents a superior view of the uterine horn in an animal used as a control, which showed adhesions over 66% of the horn surface. The group of animals treated only with the photopolymerized macromer showed an adhesion score of 13±11.4% (n=10). Of these, 4 animals showed less than 5% adhesions with only an occasional filmy band visible.

The animals treated with photopolymerized gel containing tPA showed further improved results over the "gel only" animals. One animals showed a filmy band on both the right and left horn. They were assigned a score of 5% with a total score of 10%. The other animal did not show any adhesions at all. Thus the total score for these animals was 5±5%.

Figure 5B shows normal horn anatomy in a typical horn which has undergone gel treatment. Adhesions are filmy in all cases and no dense bands are seen. No traces of the remaining gel could be observed. Typical samples of horns showing filmy adhesions showed some fibrous tissue with a 6-15 cell thick layer of fibroblasts showing some collagen fibrils but no formation of dense collagen fibers. The horns showing no adhesions occasionally showed a 1-4 cell thick layer of fibroblasts, but mostly a normal epithelium with no evidence of inflammatory cells.

This same procedure was slightly modified as described below as a better mode of using the polymers

to prevent postoperative adhesions using the rat uterine horn model.

Female rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally), and a midline laparotomy was performed. The uterine horns were exposed, and the vasculature in the arcade feeding the horns was systematically cauterized using bipolar cautery; the most proximal and most distal large vessel on each horn were not cauterized. Following this, the antimesenteric surface of each horn was cauterized at two 1 mm diameter spots on each horn, each separated by a 2 cm distance, the pair centered along the length of each horn. Following injury, 0.5 ml of macromer solution was applied per horn and was gelled by exposure to long wavelength ultraviolet light (365 nm, approximately 20 mW/cm^2) for 15 sec per surface on the front side and on the back side each. The uterus was replaced in the peritoneal cavity, and the musculoperitoneal and skin layers were closed.

The macromer consisted of a PEG chain of MW 8,000 daltons, extended on both sides with a lactic acid oligomer of an average degree of polymerization of 5 lactidyl groups, and further acrylated nominally at both ends by reaction with acryloyl chloride. In one batch, Batch A, the degree of acrylation was determined by NMR to be approximately 75%, and in another, Batch B, it was determined to be greater than approximately 95%. The macromer was dissolved in saline at a specified concentration, and the initiation system used was 2,2-dimethoxy-2-phenyl acetophenone from a stock solution in N-vinyl pyrrolidinone, the final concentration of 2,2-dimethoxy-2-phenyl acetophenone being 900 ppm and the final concentration of N-vinyl pyrrolidinone being 0.15%.

In one set of experiments, macromer from Batch A was applied in varying concentrations, and adhesions

were scored at 7 days postoperatively. Scoring was performed by two means. The length of the horns involved in adhesions was measured with a ruler, and the fraction of the total length was calculated. The nature of the adhesions was also scored on a subjective scale, 0 being no adhesions, 1 being filmy adhesions that are easily separated by hand, and 2 being dense adhesions that can only be separated by sharp instrument dissection. Furthermore, one of the samples contained tissue-plasminogen activator (t-PA), which is known to reduce adhesions, at a concentration of 0.5 mg/ml (0.5%) macromer solution. The results are shown in Table 11 for macromer batch A and batch B.

In a third set of experiments, adhesions were formed in female rats as described above, and the adhesions were surgically lysed 7 days after the initial surgery. The extent and grade of adhesions was scored during lysis. The animals were divided into two groups, and one group was treated with macromer from Batch B at a concentration of 10%. The results are shown in Table 11 as batch B, 10%.

Table 11: Reduction of Adhesions with Polymer.

Concentration macromer	Extent of adhesions % (S.D.)	Grade of adhesions (0-2)	Number of Animals
Polymer A			
15%	24.6 (3.1)	1.1 (0.1)	7
20%	33.6 (9.8)	1.2 (0.3)	7
25%	37.5 (11.1)	1.2 (0.1)	7
30%	54.2 (12.0)	1.6 (0.4)	6
20% + t-PA	18.3 (6.4)	1.1 (0.1)	6
Control (saline)	72.6 (18.7)	1.5 (0.2)	7
Polymer B			
5%	22.1 (4.2)	1.2 (0.1)	7
10%	10.0 (5.1)	1.0(0)	7
15%	17.8 (5.7)	1.0 (0)	7
20%	26.3 (11.4)	1.4 (0.2)	7
Control (saline)	75.9 (4.4)	1.8 (0.3)	7

Polymer B, 10%

Scoring performed at:	group that became:			
time of lysis	Controls	85.9 (9.7)	1.8 (0.1)	7
Time of lysis	Treatment	79.4 (6.8)	1.7 (0.2)	7
7 days post-lysis	Controls	78.8 (11.3)	1.8 (0.1)	7
7 days post-lysis	Treatment	28.2 (5.1)	1.0 (0)	7

The above results illustrate that the photopolymerized macromer can reduce or prevent post operative adhesions in both primary adhesions and adhesiolysis models, and moreover that the gel can be used to locally release a drug to exert a combined beneficial effect.

Example 13: Nerve anastomosis.

The sciatic nerve of a rat was aseptically severed using a scalpel and allowed to pull apart. The two ends of the nerve were reopposed using sterile forceps, and a 50% solution in buffer of polymer 1KL,

a macromer made from PEG 1K with lactide chain extension and acrylate termination, with 0.1% 2,2-dimethoxy-2-phenoxy acetophenone was applied to the nerve stumps. The affected area was illuminated with a 100 W LWUV lamp for 60 seconds, and an adhesive bond was observed to form between the proximal and distal nerve stumps.

To ensure the biocompatibility of the applied material with the nerve tissue, the same solution of macromer was applied to nonsevered rat sciatic nerves, and the area of the incision was closed using standard small animal surgical technique. The area was reopened at 1 hour or 24 hour postoperatively, and the affected area of the nerve was removed en block and prepared for transmission electron microscopy. No morphological differences were observable between the treated nerves at either time point as compared to control rat sciatic nerves that were otherwise nonmanipulated, even though they had been traumatized and manipulated.

Example 14: Evaluation of PEG Based Degradable Gels as Tissue Adhesives.

Abdominal muscle flaps from female New Zealand white rabbits were excised and cut into strips 1 cm X 5 cm. The flaps were approximately 0.5 to 0.8 cm thick. A lap joint, 1 cm X 1 cm, was made using two such flaps. Two different compositions, 0.6KL and 1 KL, were evaluated on these tissues. Both these compositions were viscous liquids and were used without further dilution. 125 μl of ethyl eosin solution in N-vinyl pyrrolidone (20 mg/ml) along with 50 μl of triethanolamine was added to each ml of the adhesive solution. 100 μl of adhesive solution was applied to each of the overlapping flaps. The lap joint was then irradiated by scanning with a 2 W argon ion laser for 30 sec from each side. The strength of the resulting joints was evaluated by measuring the

force required to shear the lap joint. One end of the lap joint was clamped and an increasing load was applied to the other end, while holding the joint was clamped and an increasing load was applied to the other end, while holding the joint horizontally until it failed. Four joints were tested for each composition. The 1KL joints had a strength of 6.6±1.0 KPa (mean ±S.D.), while the 0.6KL joints had a strength of 11.4±2.9KPa. It is significant to note that it was possible to achieve photopolymerization and reasonable joint strength despite the 6-8 mm thickness of tissue. A spectrophotometric estimate using 514 nm light showed less than 1% transmission through such muscle tissue.

Example 15: Coupling of Photopolymerizable Groups to Proteins (Albumin).

PEG (M.W. 2,000) monoacrylate (5g) was dissolved in 20 ml dichloromethane. Triethyl amine (0.523 g) and 2,2,2-trifluoroethanesulfonyl chloride (tresyl chloride) (0.017 g) were added and the reaction was allowed to proceed for 3 hours at 0°C under nitrogen atmosphere. The reaction mixture was then filtered and the dichloromethane evaporated to dryness. The residue was redissolved in a small amount of dichloromethane and precipitated in diethyl ether. The polymer was then filtered and dried under vacuum for 10 hours and used directly in the subsequent reaction with albumin.

1 g of bovine serum albumin was dissolved in 200 ml of sodium bicarbonate buffer at pH 9. Tresyl activated PEG monoacrylate (5 g) was added and the reaction was stirred for 24 hours at 25°C. Albumin was separated by pouring the reaction mixture into acetone. It was further purified by dialysis using a 15,000 daltons cutoff dialysis membrane. A 10% w/v solution of the PEG acrylated albumin could be photopolymerized with long wave UV radiation using 0.9

mg/ml of 2,2 dimethoxy 2 phenylacetophenone as the initiator. In this gel the degradable segment is the protein albumin.

Example 16: Modification of Polysaccharides (Hyaluronic Acid)

In a dry 250 ml round bottom flask, 10 grams of PEG 400 monomethacrylate was dissolved in 100 ml dry dioxane, to which 4.053 g of carbonyl diimidazole (CDI) was slowly introduced under nitrogen atmosphere and the flask was heated to 50°C for 6 h. Thereafter the solvent was evaporated under vacuum and the CDI activated PEG monomer was purified by dissolving in dichloromethane and precipitating in ether twice.

1 g of hyaluronic acid, 5 g of CDI activated PEG 400 monoacrylate were dissolved in 200 ml sodium borate buffer (pH 8.5) and the solution was stirred for 24 hours. It was then dialyzed using a 15,000 dalton cutoff dialysis membrane to remove unreacted PEG. A 10% w/v solution of the acrylated hyaluronic acid was photopolymerized with long wave UV radiation, using 0.9 mg/ml of 2,2-dimethoxy-2-phenylacetophenone as the initiator. In this gel, the degradable region is hyaluronic acid.

Example 17: PEG Chain Extended with Polyorthocarbonates and Capped with Urethane Methacrylate.

3, 9-bis(methylene) 2,4,8,10-tetraoxaspiro [5,5] undecane (1g) and polyethylene glycol (molecular weight, 1,000, 7.059 g) were weighed into a 250 ml Schlenk tube under dry nitrogen atmosphere in a glove bag. 50 ml of dry tetrahydrofuran was introduced under nitrogen atmosphere and reaction mixture was stirred for 6 hours at 50°C. This is a typical step growth reaction with a disturbed stoichiometry, resulting in low molecular weight poloyorthocarbonate with terminal hydroxy groups. The oligomer was separated by precipitating in hexane and dried under

vacuum. 5 g of oligomer was redissolved in dry THF to which 20 μl of dibutyltindilaurate and 2 ml of 2-isocyanatoethyl methacrylate were slowly introduced and temperature was raised to 50°C. It was held there for 6 hours and cooled. The product was separated by precipitation in hexane. In this gel, the degradable region is a polyorthocarbonate.

Example 18: Microencapsulation of Animal Cells.

A 23% w/w solution of 18.5KG in HEPES buffered saline (5 ml) was used to resuspend 10^6 CEM-SS cells. Ethyl eosin (10^{-4} M) was used as a solution in N-vinyl pyrrolidone as the initiator and triethanolamine (0.01 M) was used as the coinitiator. The solution was then exposed through a coextrusion apparatus to an argon ion laser (514 nm, 2 Watts). The coextrusion apparatus had mineral oil as the fluid flowing annularly (flow rate 4 ml/min) around an extruding stream of the precursor cell suspension (flow rate 0.5 ml/min). The microdriplets gelled rapidly on being exposed to the laser light and were collected in a container containing PBS. The oil separated from the aqueous phase and the microspheres could be collected in the PBS below. The microspheres formed were thoroughly washed with PBS buffer to remove unreacted monomer and residual initiator. The size and shape of microspheres was dependent on extrusion rate and extruding capillary diameter (18 Ga to 25 Ga). The polymerization times were dependent on initiator concentration (ethyl eosin 5 μM to 0.5 mM, vinyl pyrrolidone (0.001% to 0.1%), and triethanolamine (5 mM to 0.1 M), laser power (120 mW to 2W), and monomer concentration (>10%w/v). Spheres prepared using this method had a diameter from 500 μm to 1,200 μm. The polymerizations were carried out at physiological pH in the presence of air. This is significant since radical polymerizations may be affected by the presence of oxygen. Cell viability subsequent to

encapsulation was checked by trypan blue exclusion assay and the encapsulated cells were found to be more than 95% viable after encapsulation.

Example 19: Various Formulations for the Prevention of Post Operative Adhesions.

The utility of PEG-oligo(α-hydroxy acid) diacrylates and tetraacrylates to prevent postoperative adhesions was evaluated in the rabbit uterine horn model as described above. The following polymers were synthesized, as described above: PEG 6K lactide diacrylate (6KL), PEG 10K lactide diacrylate (10KL). PEG 18.5K lactide (18.5KL), PEG 20K lactide (20KL). Solutions with 24% polymer in PBS with 900 ppm 2,2-dimethoxy-2-phenyl acetophenone, were prepared as described above. The solutions were applied to the uterine horn after cautery of the vascular arcade and illuminated with a 365 nm LWUV lamp, as described above. In one formulation, 18.5KL, 5 mg t-PA was mixed into the solution before application. Controls consisted of animals manipulated and cauterized but not treated with macromer solution. Measurement was performed on the 14th ± 1 day. Extent of adhesion was estimated from the fraction of the horn that was involved in adhesions, and the tenacity of adhesions was scored as 0, no adhesions; 1, filmy adhesions that offer no resistance to dissection; 2, fibrous adhesions that are dissectable by hand; 3, fibrous adhesions that are dissectable by blunt instruments; and 4, fibrous adhesions that are dissectable by sharp instruments. The results were as follows, where the extent of adhesions and the tenacity of the adhesions are shown.

Table 12: Efficacy of Polymer in Preventing Adhesions.

Formulation	Number of animals	Extent, %, ± S.D.	Tenacity, 0-4 ± S.D.
6KL	7	0.9 ± 1.7	0.9 ± 0.7
10KL	7	0 ± 0	0 ± 0
20KL	6	4.4 ± 5.0	0.9 ± 0.7
18.5KL t-PA	7	8.9 ± 13.1	1.6 ± 1.3
Control	7	35 ± 22	3.3 ± 0.6

Example 20: Polymerization of Ultrathin layers of Polymer on the surface of blood vessels to reduce thrombosis after vessel injury.

Blood vessels were harvested from rats and were rinsed free of blood. The endothelium of the vessel were removed by inserting a wooden dowel and rotating the vessel over the dowel. One vessel was used as a control, and was exposed to flowing blood as described below without further modification. Another vessel was treated first by exposure to eosin Y at 1 mM in saline, then rinsed in HEPES buffered saline, then filled with a solution of PEG-MA, PEG 10K with acrylate end-capped oligomers of DL lactide, containing triethanolamine (TEA) (100 mM) and N-vinylpyrrolidone (VP) (0.15%) and then illuminated by exposure to an argon ion laser at 0.5 W/cm2 for 15 sec. The nonpolymerized prepolymer mixture in the lumen of the vessel was rinsed away with saline. Human blood was collected from the antecubital vein and was anticoagulated with heparin at 2 units/ml. This blood was perfused through each vessel by a syringe pump at a flow rate corresponding to a wall shear rate of approximately 200/s for 7 min. The vessel was then superficially rinsed in saline and fixed in formaldehyde.

The treated vessel did not appear colored or different in color after perfusion compared to its color before perfusion, while the untreated control vessel appeared blood red. Thin segments of each vessel were cut from each vessel, were mounted on end, and were examined by environmental scanning electron microscopy (ESEM). ESEM is performed on hydrated samples in relatively low vacuum. This permits the visualization of the polymer film coating in the swollen and wet state. This is important to obtain measurements that may be readily interpreted, since the polymer film is approximately 95% water. A high degree of thrombosis was readily observed in the control vessel. The lumen of this vessel was narrowed to less than one-third its diameter pre-perfusion by the accumulation of thrombus, as shown in Figure 6A. By contrast, no thrombus could be observed in the lumen of the treated vessel, as shown in Figure 6B. A higher magnification of the vessel wall demonstrated no adherent thrombus. A still higher magnification shows a white structure which is the polymer film, which is different in contrast from the tissue due to differential charging under the electron beam of the ESEM. The film may be seen to be precisely conformed to the shape of the vessel and be approximately 5 - 8 μm thick.

The region of polymerization was restricted to the neighborhood of the blood vessel wall surface. The photosensitive dye was adsorbed to the vessel wall. Unbound dye was rinsed away. The entire lumen was filled with prepolymer, but upon illumination the gel formation was restricted to the vessel wall where the dye and the prepolymer meet. This interfacial polymerization process can be conducted to produce surface adherent layers that vary in thickness from less than 7 μm to more than 500 μm.

The above procedure was performed in 8 control rat arteries, and 8 treated arteries, with equivalent light microscopic histological results as described above. As demonstrated by this study, PEG prepolymers can be polymerized upon the lumenal surface of blood vessels. The immediate effect of this modification is to reduce the thrombogenicity of an injured blood vessel surface. This has clear utility in improving the outcome of balloon angioplasty by reducing the thrombogenicity of the vessel and lesion injured by balloon dilation. Another effect of this modification is to be reduce smooth muscle cell hyperplasia. This may be expected for two reasons. First, platelets contain a potent growth factor, platelet-derived growth factor (PDGF), thought to be involved in post-angioplasty hyperplasia. The interruption of the delivery of PDGF itself poses a pharmacological intervention, in that a "drug" that would have been delivered by the platelets would be prevented from being delivered. Thrombosis results in the generation of thrombin, which is a known smooth muscle cell mitogen. The interruption of thrombin generation and delivery to the vessel wall also poses a pharmacological intervention. There are other growth factors soluble in plasma which are known to be smooth muscle cell mitogens. The interruption of thrombin generation and delivery to the vessel wall also poses a pharmacological intervention. Moreover, there are other growth factors soluble in plasma which are known to be smooth muscle cell mitogens. The gel layer is known to present a permselective barrier on the surface of the tissue, and thus the gel layer may reasonably be expected to reduce hyperplasia after angioplasty. The inhibition of thrombosis upon the vessel wall may also reduce the incidence of abrupt reclosure and vasospasm, both of which occur sometimes following vascular intervention.

Example 21: Interfacial Polymerization of Macromers Inside Blood Vessels to Prevent Thrombosis.

Macromer solutions were polymerized interfacially within previously injured blood vessels *in vivo* to prevent thrombosis. The carotid artery was exposed, and a polyethylene tube (PE-10) was used to cannulate the exterior carotid artery. The artery was clamped with fine arterial clamps proximal to the interior/exterior carotid artery bifurcation and approximately 2 cm distal to the bifurcation. A 1 ml tuberculin syringe was used to rinse the blood from the lumen of the isolated zone by filling and emptying the vessel zone. The vessel was injured by crushing using a hemostat. The isolated zone was filled with a 10 mM solution of eosin Y for 2 minutes, after which it was rinsed and filled with a 20% solution of a macromer in saline with 0.1 mM triethanolamine and 0.15% N-vinyl pyrrolidinone. The macromer consisted of a PEG chain of MW 8,000 daltons, extended on both sides with a lactic acid oligomer of an average degree of polymerization of 5 lactidyl groups, and further acrylated nominally at both ends by reaction with acryloyl chloride. The vessel was illuminated transmurally using an argon ion laser (514 nm) at an intensity of approximately 1 mW/cm^2 for 5 seconds. Following this, the cannula was removed from the exterior carotid artery and the artery was ligated at the bifurcation. The arterial clamps were removed to permit the resumption of blood flow. Perfusion was allowed for 20 minutes, following which the vessel were again isolated, removed from the body, gently rinsed, fixed, and prepared for light microscopic histological analysis. Using the naked eye, the crushed segments in control animals, which lacked illumination, were red, indicating internal thrombus with entrapped red blood cells. By contrast, no

redness was observed at the site of the crush injury in the treated vessels. Histology showed extensive thrombus, fibrin, and entrapped red blood cells in the non-treated vessels. By contrast, no thrombus or fibrin or entrapped red blood cells were observed in the treated vessels. The procedure was conducted in four control animals and three treated animals.

This example demonstrates that the polymerization can be carried out *in situ* in the living animal, that the polymer coating remains adherent to the vessel wall during arterial blood flow, and that the polymer coating can prevent thrombosis *in vivo* in non-anticoagulated animals. This approach to treatment has clear benefits in preventing abrupt reclosure, vasospasm, and restenosis after intravascular interventional procedures. Moreover, it is more generally applicable to other intraluminal and open-surface organs to be treated.

Modifications and variations of the present invention, the macromer and polymeric compositions and methods of use thereof, will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the appended claims.

We claim:

1. A biodegradable, polymerizable, and at least substantially water soluble macromer comprising at least one water soluble region, at least one degradable region, and at least two free radical polymerizable regions, wherein the polymerizable regions are separated from each other by at least one degradable region.

2. The macromer of claim 1 wherein the water soluble region is attached to a degradable region, at least one polymerizable region is attached to the water soluble region, and at least one polymerizable region is attached to the degradable region.

3. The macromer of claim 1 wherein the water soluble region forms a central core, at least two degradable regions are attached to the core, and at least two polymerizable regions are attached to the degradable regions.

4. The macromer of claim 2 wherein the degradable region is a central core, at least two water soluble regions are attached to the core, and at least one polymerizable region is attached to each water soluble region.

5. The macromer of claim 1 wherein the water soluble region is a macromer backbone, the degradable region is a branch or graft attached to the macromer backbone, and at least two polymerizable regions are attached to the degradable regions.

6. The macromer of claim 1 wherein the degradable region is a macromer backbone, the water soluble region is a branch or graft attached to the degradable backbone, and two or more polymerizable regions are attached to the water soluble branches or grafts.

7. The macromer of claim 1 wherein the water soluble region is a star backbone, the degradable region is a branch or graft attached to the water

soluble star backbone, and at least two polymerizable regions are attached to a degradable branch or graft.

8. The macromer of claim 1 wherein the degradable region is a star backbone, the water soluble region is a branch or graft attached to the degradable star backbone, and two or more polymerizable regions are attached to the water soluble branch or graft.

9. The macromer of claim 1 wherein the water soluble region is also the degradable region and two or more polymerizable regions are attached to the water soluble regions.

10. The macromer of claim 1 wherein the water soluble region is also the degradable region, one or more additional degradable regions are grafts or branches upon the water soluble region, and a total of two or more polymerizable regions are attached to the degradable regions.

11. The macromer of claim 1 comprising a core, at least two extensions on the core, and an end cap on at least two extensions, wherein

the core comprises poly(ethylene glycol).

12. The macromer of claim 11 wherein

each extension comprises biodegradable poly(hydroxy acid); and

each end cap comprises an acrylate oligomer or monomer.

13. The macromer of claim 12 wherein the poly(ethylene glycol) has a molecular weight between about 400 and 30,000 Da;

the poly(hydroxy acid) oligomers have a molecular weight between about 200 and 1200 Da; and

the acrylate oligomer or monomer have a molecular weight between about 50 and 200 Da.

14. The macromer of claim 1 wherein the polymerizable regions contain a carbon-carbon double

bond capable of cross-linking and polymerizing macromers.

15. The macromer of claim 1 wherein crosslinking and polymerization of the macromer are initiated by a light-sensitive free-radical polymerization initiator with or without a cocatalyst, further comprising a free radical polymerization initiator.

16. The macromer of claim 15 wherein the initiator is selected from the group consisting of xanthine dyes, acridine dyes, thiazine dyes, phenazine dyes, camphorquinone dyes, and acetophenone dyes.

17. The macromer of claim 16 wherein the initiator is selected from the group consisting of an eosin dye with triethanolamine, 2,2-dimethyl-2-phenyl acetophenone, and 2-methoxy-2-phenyl acetophenone.

18. The macromer of claim 1 wherein crosslinking or polymerizations are initiated *in situ* by light having a wavelength of 320 nm or longer.

19. The macromer of claim 1 wherein the degradable region is selected from the group consisting of poly (hydroxy acids), poly(lactones), poly(amino acids), poly(anhydrides), poly(orthoesters), poly(phosphazines), and poly(phosphoesters).

20. The macromer of claim 19 wherein the degradable region is a poly(α-hydroxy acid) selected from the group consisting of poly(glycolic acid), poly(DL-lactic acid) and poly(L-lactic acid).

21. The macromer of claim 19 wherein the poly(lactone) is selected from the group consisting of poly(ε-caprolactone), poly (δ-valerolactone) or poly(λ-butyrolactone).

22. The macromer of claim 1 wherein the water soluble region is selected from the group consisting of poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(vinylpyrrolidone),

poly(ethyloxazoline), poly(ethylene oxide)-co-poly(propyleneoxide) block copolymers, polysaccharides, carbohydrates, proteins, and combinations thereof.

23. The macromer of claim 1 further comprising biologically active molecules selected from the group consisting of proteins, carbohydrates, nucleic acids, organic molecules, inorganic biologically active molecules, cells, tissues, and tissue aggregates.

24. A method of forming a polymeric, biocompatible material on tissue comprising

applying to the tissue a solution of biodegradable, polymerizable, and at least substantially water soluble macromer comprising at least one water soluble region, at least one degradable region, and at least two free radical polymerizable regions, wherein the polymerizable regions are separated from each other by at least one degradable region, in the presence of a free radical initiator, and polymerizing the macromer.

25. The method of claim 24 wherein the tissue is coated to prevent adhesion of the tissue to other tissue.

26. The method of claim 24 wherein the tissue is coated and adhered to other tissue during polymerization.

27. The method of claim 24 further comprising providing with the macromer solution biologically active molecules selected from the group consisting of proteins, carbohydrates, nucleic acids, organic molecules, inorganic biologically active molecules, cells, tissues, and tissue aggregates.

28. The method of claim 24 further comprising first applying a free radical initiator at the site where the macromer solution is to be polymerized.

29. The method of claim 28 wherein the initiator binds to the tissue, further comprising

removing unbound initiator prior to application of the macromer solution.

30. A method for controlled release of biologically active molecules comprising mixing biologically active molecules with a solution of biodegradable, polymerizable, and at least substantially water soluble macromer comprising at least one water soluble region, at least one degradable region, and at least two free radical polymerizable regions, wherein the polymerizable regions are separated from each other by at least one degradable region, in the presence of a free radical initiator, and polymerizing the macromer to entrap the molecules within the resulting polymer.

31. The method of claim 30 wherein the polymer forms a shape selected from the group consisting of microspheres, sheets, rods, and particles.

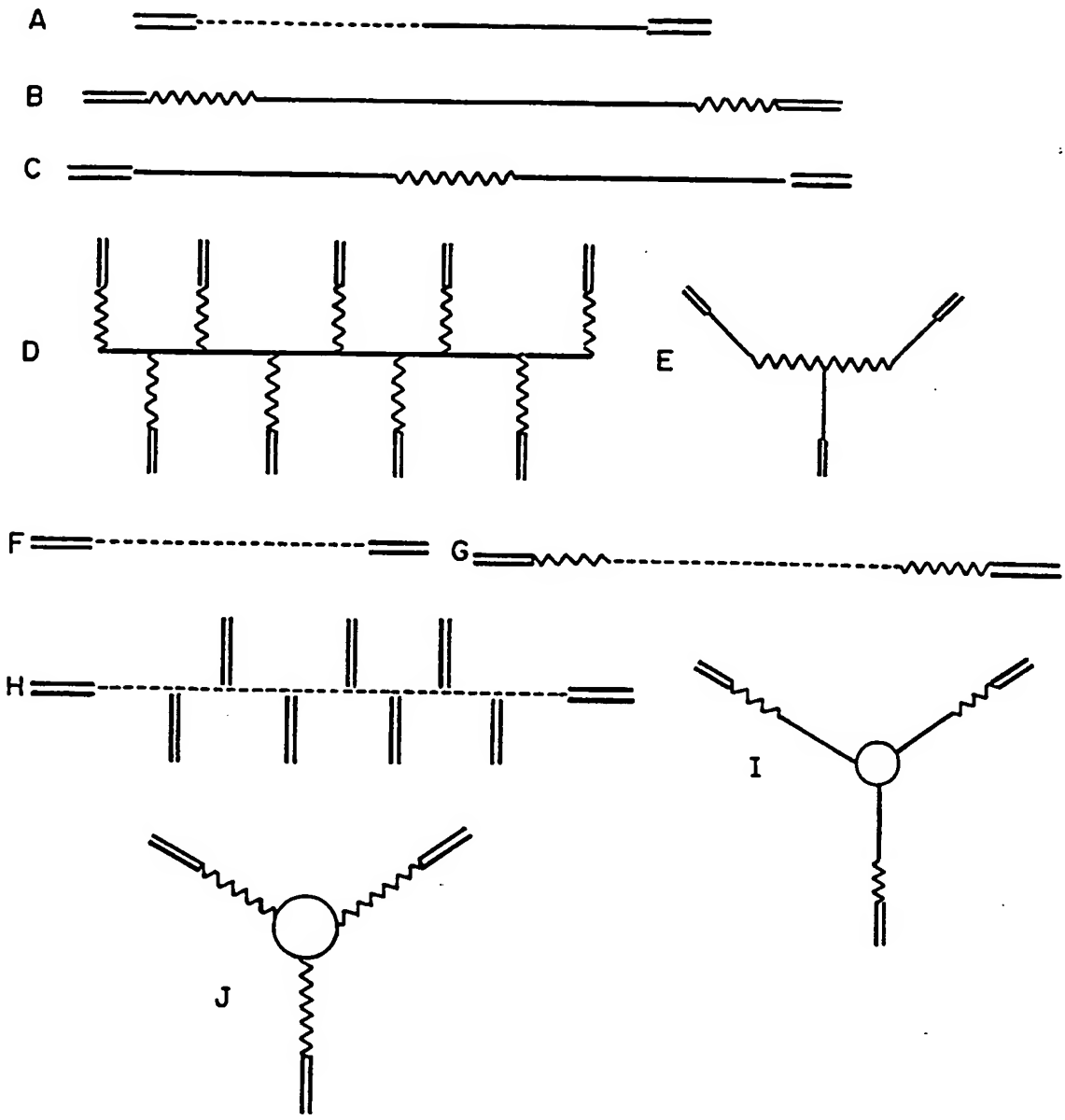
A
B
C
D
E
F
G
H
I
J
- WATER SOLUBLE COMPONENT
- HYDROLIZABLE COMPONENT
- WATER SOLUBLE AND HYDROLIZABLE COMPONENT
- PHOTOPOLYMERIZABLE COMPONENT

FIG. 1

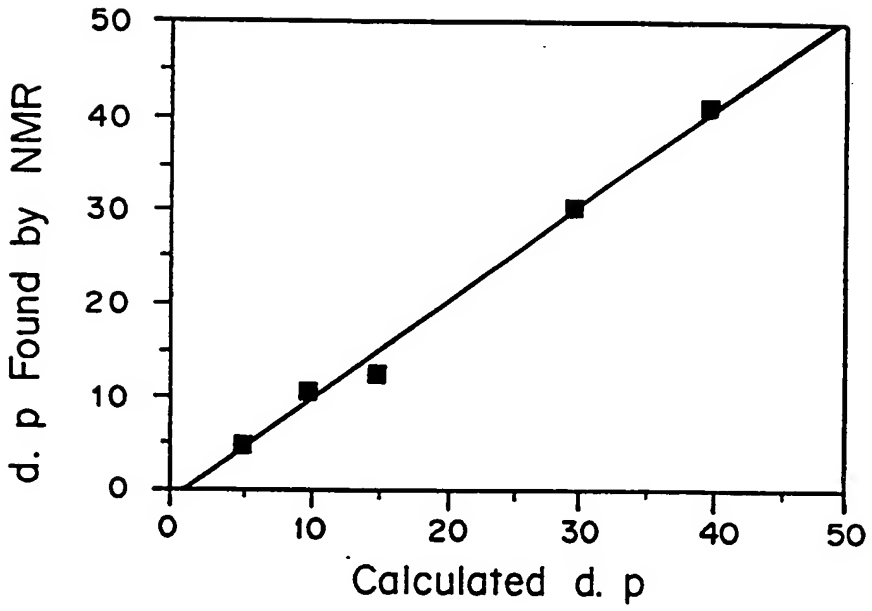

FIG. 1a

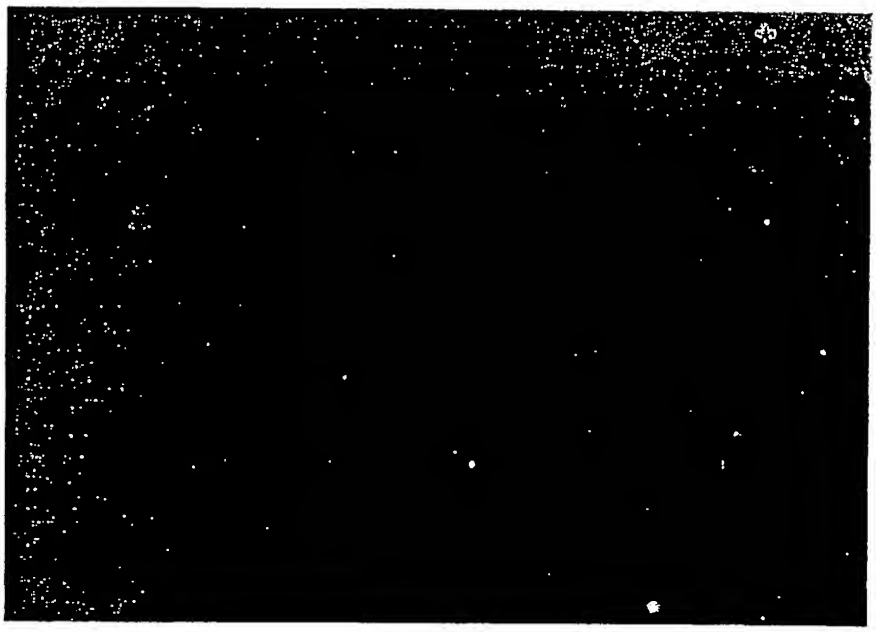

FIG. 2a

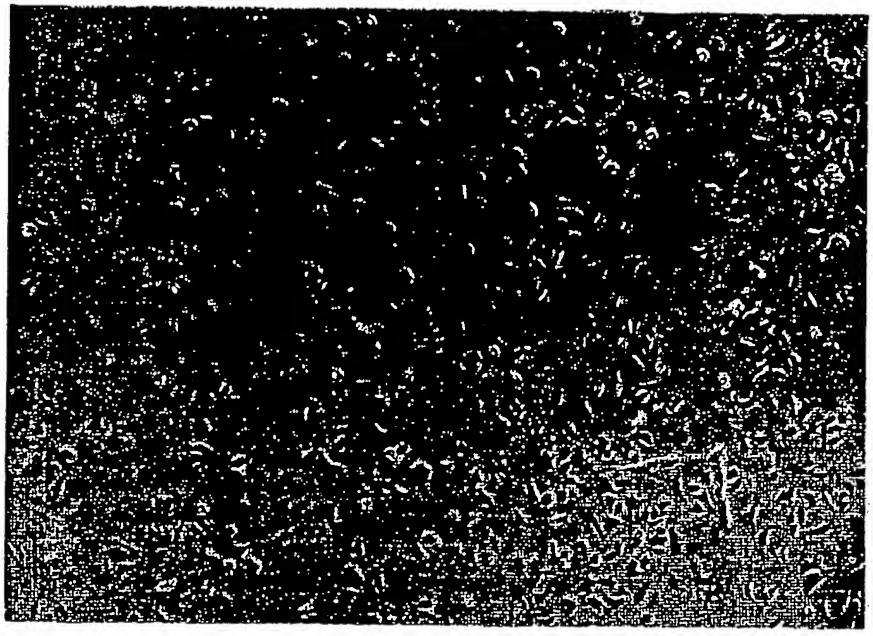

FIG. 2b

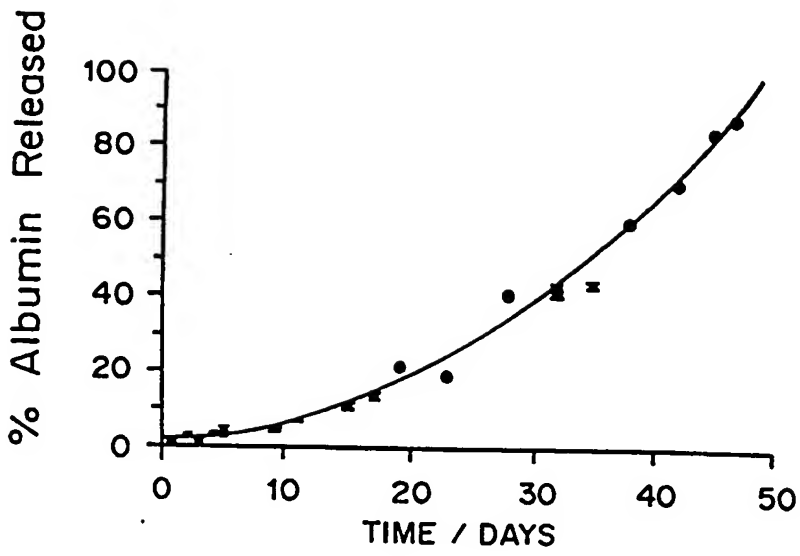
% Albumin Released
100
80
60
40
20
0
0
10
20
30
40
50
TIME / DAYS

FIG. 3a

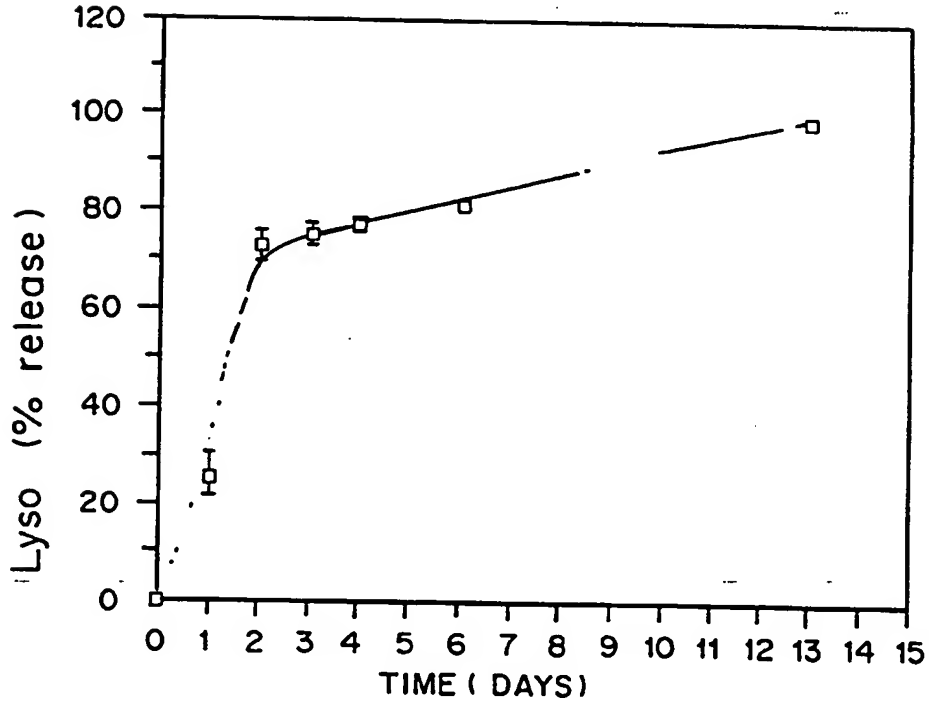
Lyso (% release)
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (DAYS)

FIG. 3b

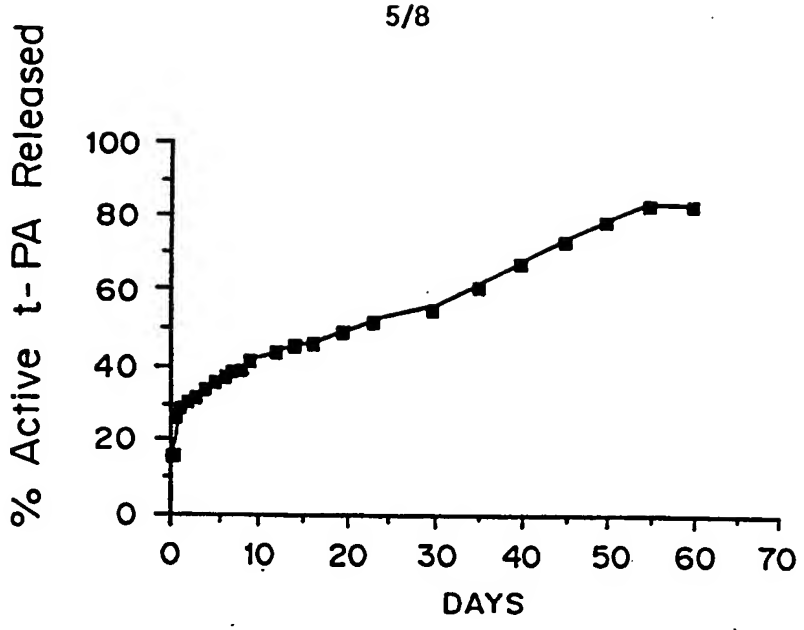
% Active t-PA Released
100
80
60
40
20
0
0
10
20
30
40
50
60
70
DAYS

FIG. 4a

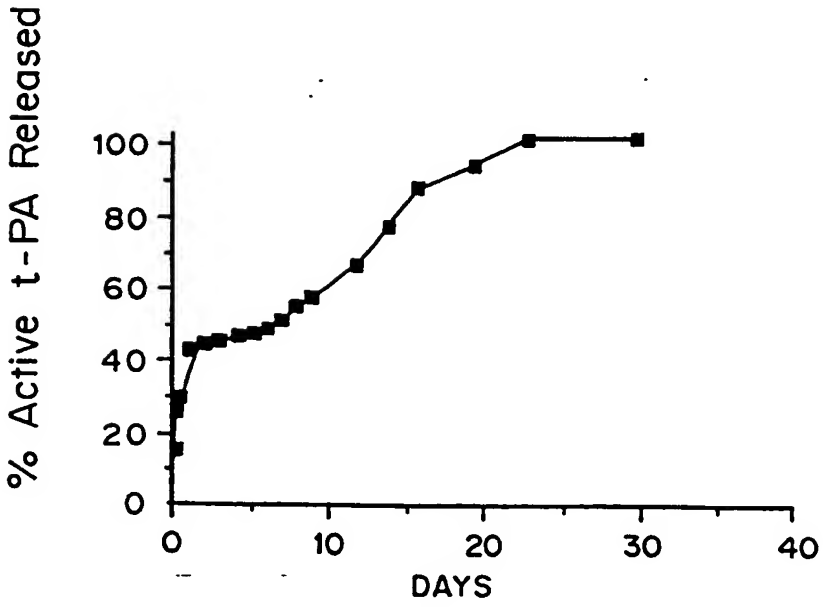
% Active t-PA Released
100
80
60
40
20
0
0
10
20
30
40
DAYS

FIG. 4b

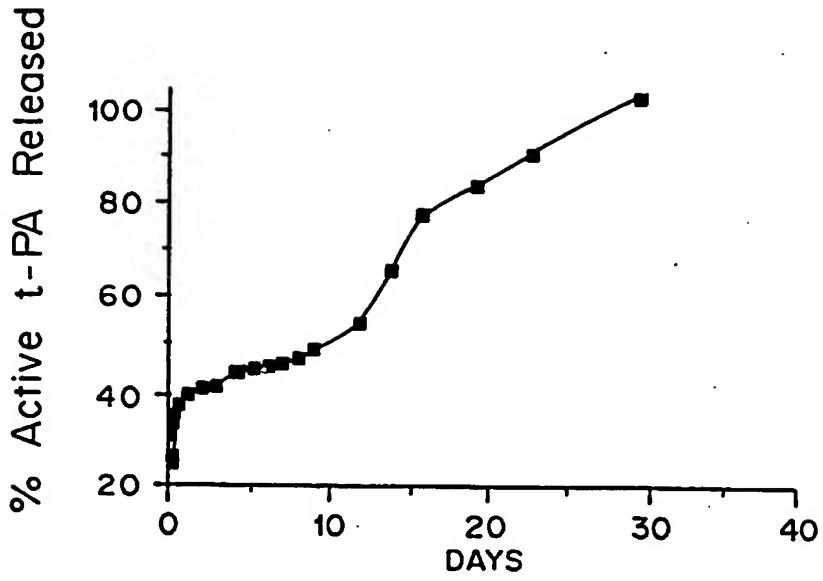

FIG. 4c

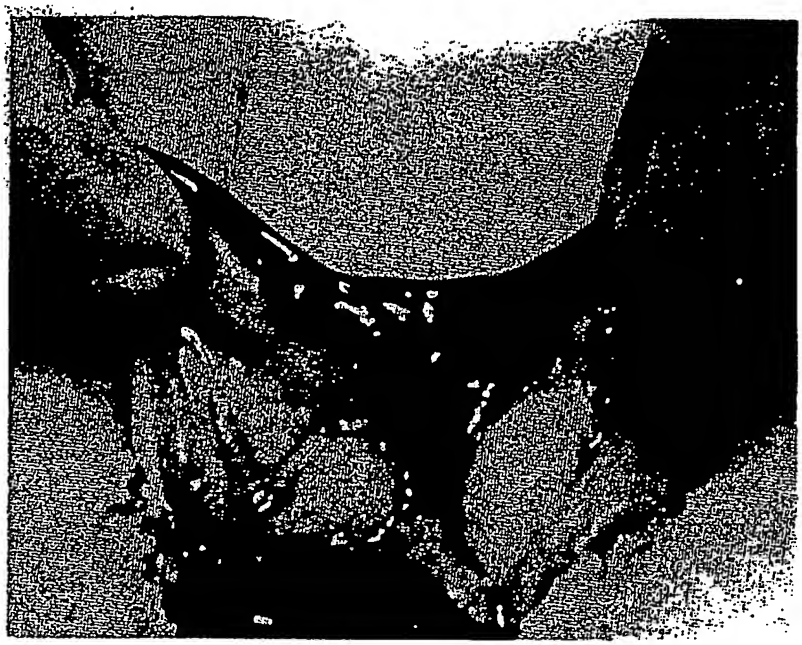

FIG. 5a

FIG. 5b

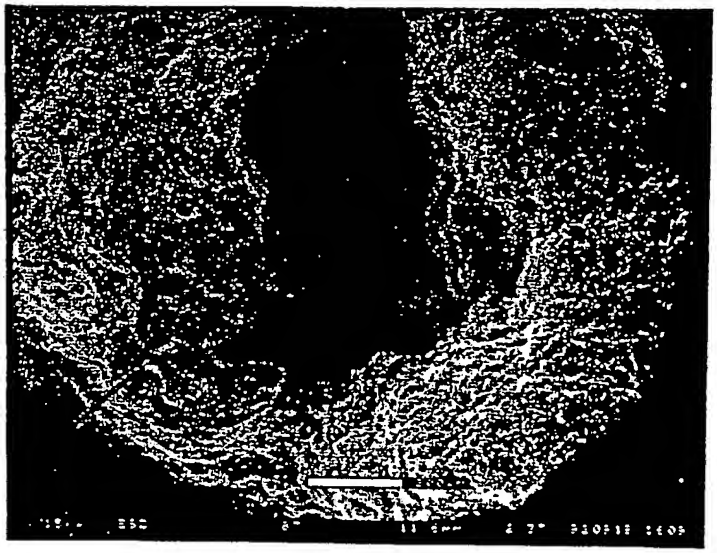

FIG. 6a

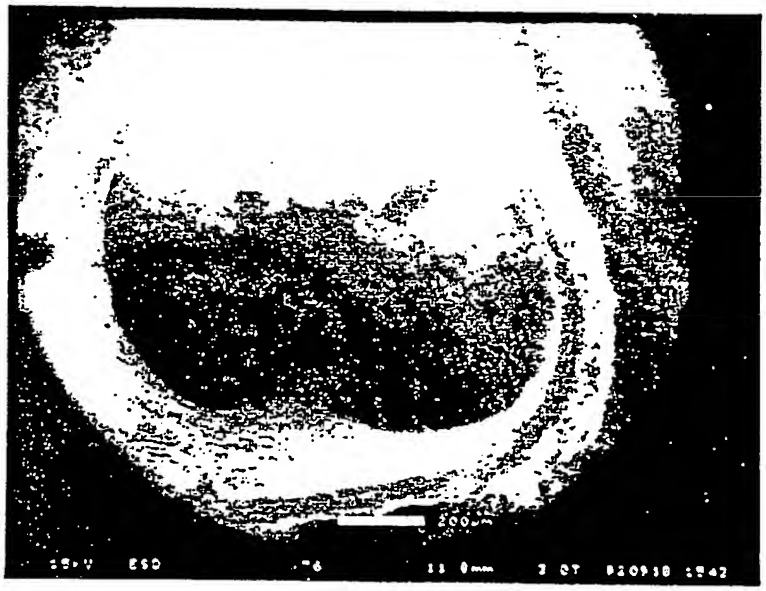

FIG. 6b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01773

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 9/50
US CL :522/26, 43, 56, 68, 87, 88, 149, 171, 181; 424/423, 426, 489
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 522/26, 43, 56, 68, 87, 88, 149, 171, 181; 424/423, 426, 489

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,310,397 (KAETSU ET AL) 12 JANUARY 1982. See entire document.	1-31
A	US, A, 4,994,277 (HIGHAM ET AL.) 19 FEBRUARY 1991. See entire document.	24-31
A,P	US, A, 5,135,751 (HENRY ET AL.) 04 AUGUST 1992. See entire document.	24-31
A,P	US, A, 5,108,755 (DANIELS ET AL.) 28 APRIL 1992. See entire document.	24-31
A	US, A, 4,999,417 (DOMB) 12 MARCH 1991 See entire document.	24-31

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
04 APRIL 1993

Date of mailing of the international search report
08 AUG 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer
AMY HULINA

Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01773

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation f document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,512,910 (SCHMIDLE) 23 APRIL 1985 See entire document.	1-23
A	US, A, 4,533,445 (ORIO) 06 AUGUST 1985 See entire document.	1-23